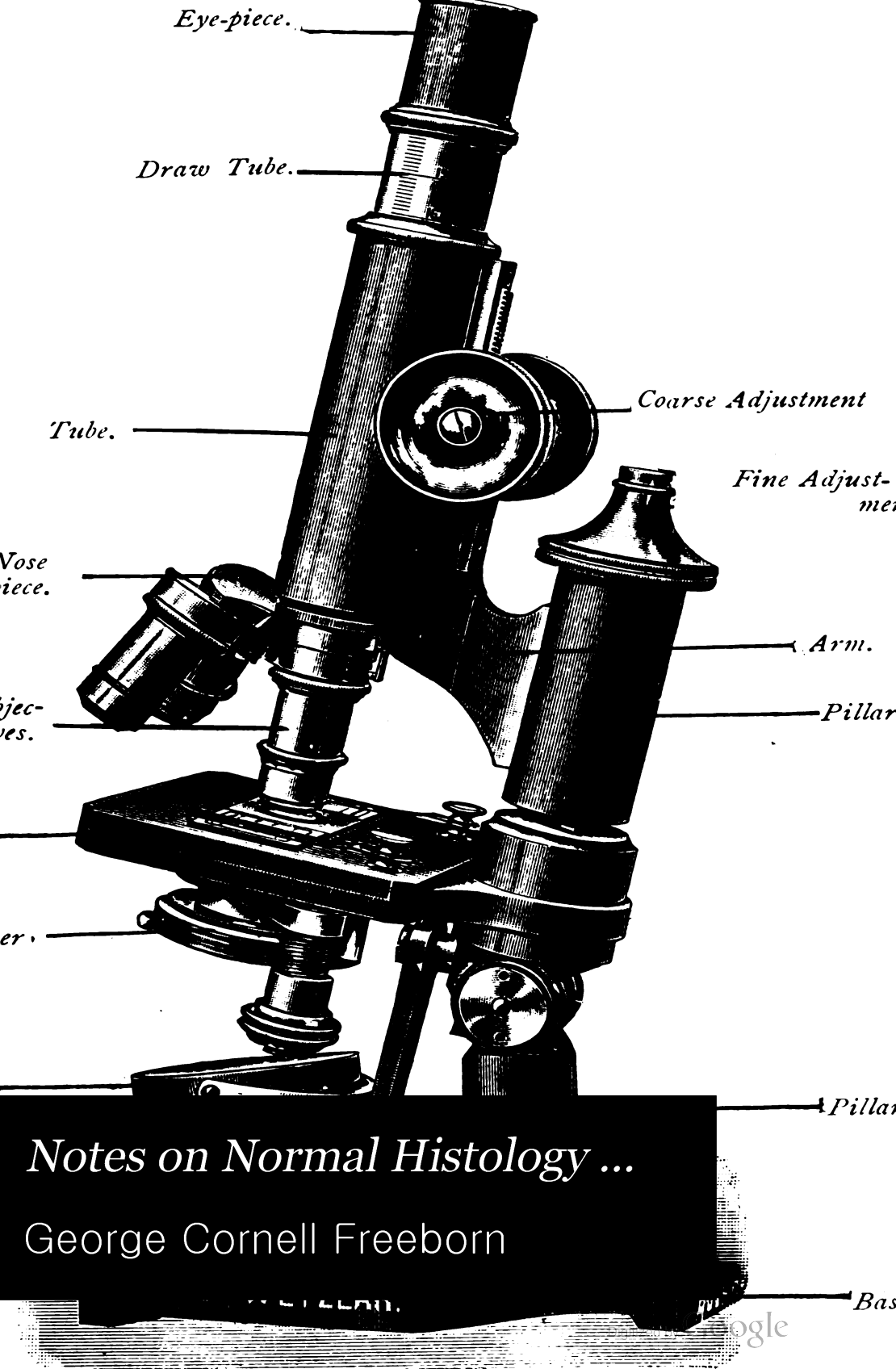

Notes on Normal Histology ...

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NOTES

ON

Normal Histology

BY

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FOR SALE BY

J. T. DOUGHERTY, BOOKSELLER

409 & 411 WEST 59TH ST.

NEW YORK

Presss of STETTINER BROS.,
52 DUANE STREET,
NEW YORK.

PART FIRST.

The Microscope and General Technique.

THE MICROSCOPE.

The microscope (Fig. 1) consists of the **mechanical parts or stand** and the **optical parts,** *objectives, eye-pieces, condenser,* and *mirror.*

Mechanical Parts.

Base.—The base is usually horseshoe in shape. It should be heavy and must rest evenly on the table.

Pillar.—The pillar rises vertically from the back part of the base. Sometimes it is in one piece, but usually it is divided into two parts by a hinge.

Stage.—The stage is attached to the pillar, at right angles, just above the hinge. It is rectangular in shape and firmly fixed to the pillar by the arm. In its centre there is a circular opening to permit of the front lens of the condenser to come up to a level with its surface. The object for examination, mounted on a slide, is placed on it.

Tube.—The tube is connected with the arm and through this with the upper part of the pillar. The *eye-piece,* or *ocular,* fits into its upper end and the *nose-piece* screws into its lower end.

Draw Tube.—The draw tube is fitted into the upper end of the tube and slides either upward or downward, thus enabling the length of the tube to be regulated. On the outside of the draw tube is engraved a millimetre scale, by which the length of the tube can be measured.

Coarse Adjustment.—The coarse adjustment consists of a rack and pinion. The rack is attached to the posterior surface of the tube. The pinion, which is turned by the large milled heads fastened to the end of the axle, is situated in the front part of the arm. The teeth on the pinion work in the rack, so that upon

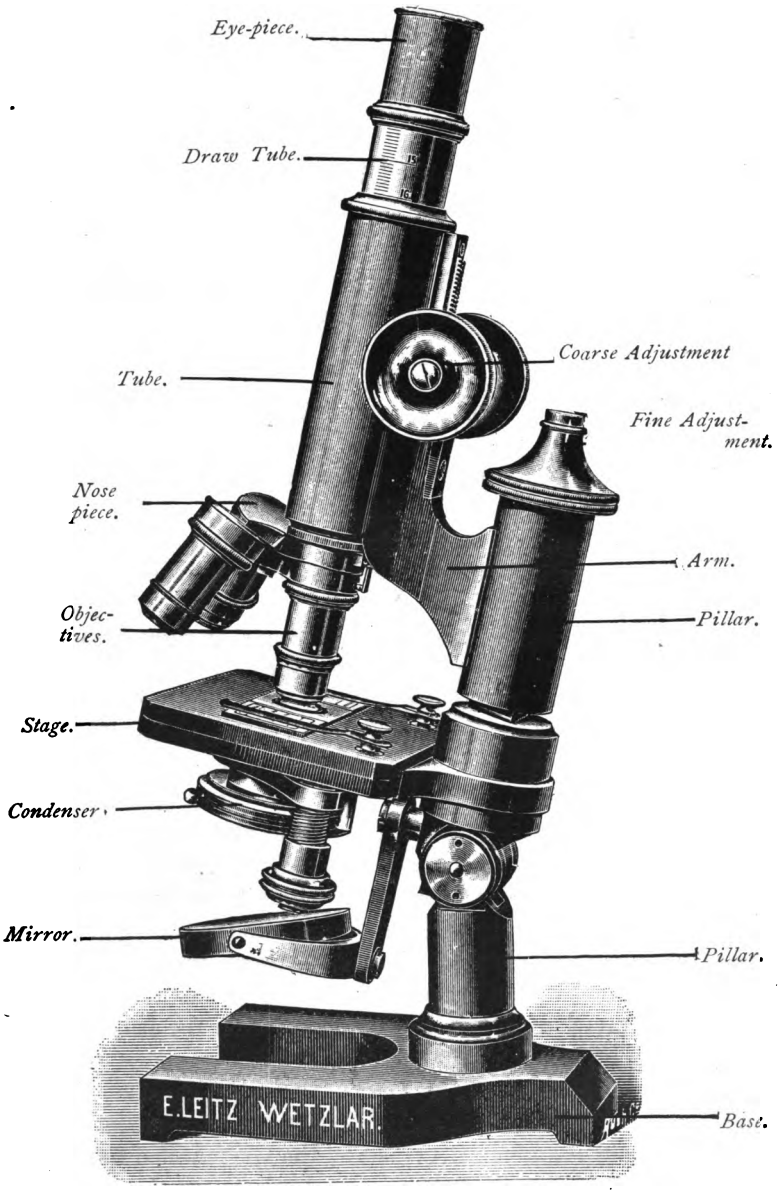


FIG. 1.—THE MICROSCOPE.

turning the milled heads the tube is caused to move up or down. This adjustment is for the purpose of **approximate focussing** only.

Fine Adjustment.—The fine adjustment is situated on the head of the pillar. It consists of a steel screw, with 50 threads to the inch (micrometer screw), fixed in the conical milled head. The screw, working on a fixed point on the head of the pillar, causes the arm and tube to move upward or downward. On account of the fineness of the screw the motion is slow. This adjustment is used to bring the object into **sharp focus, and must never be used to do the work of the coarse adjustment.**

Nose-piece.—The nose-piece may be either double or triple, holding two or three objectives. It is screwed into the lower end of the tube, the objectives being screwed into its arms. It revolves so that an objective may be brought under the tube, thus doing away with the changing of them by hand.

Optical Parts.

The optical parts consist of the various glass lenses and mirrors set in frames of metal.

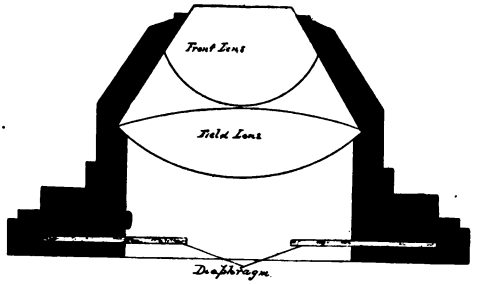


Fig. 2.—Section through Abbe Condenser.

Mirror.—The mirror is attached below the stage and is used for reflecting the light up through the condenser for illumination of the object under examination. It is constructed of silvered glass and is set in a metal frame, arranged so that it may be turned at various angles. It has two surfaces, one being **plane,** the other **concave.** The **plane surface** is the one to be used with the condenser.

Condenser.—The condenser used is that known as the Abbe. It is placed below the stage, in the **substage,** and it condenses the rays of light reflected from the mirror and brings them to a focus on the specimen placed on the stage.

It consists of two lenses (Fig. 2) of glass set in a metal frame.

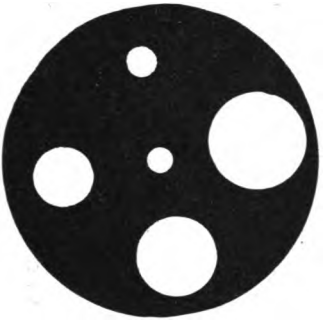


FIG. 3.—WHEEL DIAPHRAGM

The upper or front lens is a plano-convex one, the plane surface being on a level with the stage when the condenser is in proper position. The lower or field lens is biconvex, the upper surface being of a much lesser curvature than the lower.

Fitted in the lower part of the condenser is the **diaphragm,** used

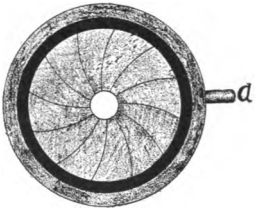


FIG. 4.—IRIS DIAPHRAGM.

for regulating the amount of light admitted to the condenser. The diaphragm is of two forms, the **wheel diaphragm** and the **iris diaphragm.** The wheel diaphragm (Fig. 3) is a circular plate of metal, near the circumference of which are different-sized circular openings. It turns on a central axis, thus permitting the proper-

sized opening to be brought under the centre of the condenser. The iris diaphragm (Fig. 4) is formed of a series of overlapping plates or shutters of steel set in a circular frame. By moving the knob *a*, on the side of the condenser, these plates move over each other, enlarging or narrowing the central opening.

Ocular, or **Eye-piece.**—The ocular, or eye-piece, fits into the upper end of the draw tube. It consists of two plano-convex lenses (Fig. 5), the **field lens** and the **eye lens.** The form used is the negative or Huygenian, and it gives an *inverted* picture of the object under examination.

The eye-piece magnifies the image formed by the objective. They are of various magnifying powers, their magnification vary-

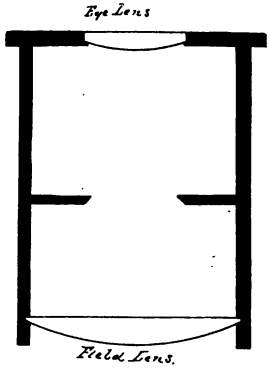


FIG. 5.—OCULAR, OR EYE-PIECE.

ing from 5 to 20 diameters. The high powers are short, the low powers long. Their value is indicated by the letters of the alphabet, A, B, C, etc., or by the numerals I, II, III, etc., engraved on them. The first letters and the low numbers indicate low powers.

Objectives, or **Lenses.**—The objective, or lens, consists of a series of systems of lenses of glass set in a tube of metal; **low powers** having two systems, **high powers** three or more. The pair of lenses nearest the object is the **front lens,** that furthest away the **back lens.**

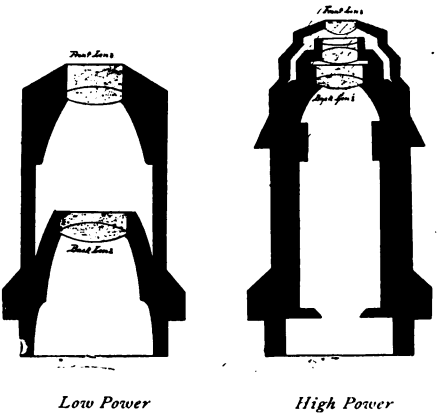
High and Low Powers.—The low-power lenses have a magnifying power of from 5 to 90 diameters, high powers from 100 to 500 diameters or more. The diameter of the front lens of the low

powers is much larger than that of the high powers; in some of the latter it may not be larger than a pinhead.

The magnifying power of the objectives is indicated by figures or their focal length being engraved on them. Thus, a No. 2 or 3, or a two-thirds of an inch, or 18 mm. is a low power; a No. 7, one-fifth of an inch, or 4 mm. objective is a high power.

The magnifying power of the objectives and eye-pieces used by us, is for the low power 70 to 90 diameters; for the high power, about 450 diameters.

Focal Distance.—The focal distance of a lens is the equivalent of a simple biconvex lens whose focus is the same as that of the



Low Power *High Power*

FIG. 6.—OBJECTIVES OR LENSES

objective. For example, a one-fifth of an inch or 4 mm. objective produces the same sized image as a simple biconvex lens whose focus is one-fifth of an inch or 4 mm.

Working Distance.—The working distance of an objective is the distance between the front lens and the object when the latter is in focus. It varies with the different powers, decreasing with the magnification, and is always less than the focal distance.

Tube Length.—All modern objectives are corrected for a certain tube length. This varies with the objectives of different makers. The Leitz objectives, used by us, are corrected for a tube length of 170 mm. without the nose-piece, and 160 mm. with it.

An increase of the tube length increases the magnifying power, but destroys the definition of the objective, this being especially the case with the high powers.

Manipulation of the Microscope.

The microscope should rest firmly on the table in front of and a little to the left of the worker. The pillar should be toward him, and should always be vertical, thus giving a horizontal stage.

Illumination.—The light should be taken from the upper part of the window. The microscope having been placed in the proper position, you are to turn on the low-power lens, place it under the lower end of the tube by revolving the nose-piece, then place the right eye over the eye-piece; then with the right hand turn the mirror until the light received on it is reflected up through the condenser and objective, and gives a clear, round **field** without any shadows.

Focussing.—The mounted object (see page 24) is placed on the stage over the central opening. Place the right eye over the eye-piece; grasp one of the milled heads of the **coarse adjustment** with the thumb and index finger, and by turning it slowly move the tube down until the object appears in the field cloudy and indistinct. It is now in **approximate focus.** Next take hold of the head of the **fine adjustment** with the thumb and index finger of the right hand, and turn it slowly to the right or left until the outlines of the specimen become sharp and distinct. The object is now in **focus.**

Next turn your attention to the illumination. A large amount of light thrown through the specimen obliterates all detail of structure and at the same time dazzles the eye. The amount of light must be just enough to bring out the details of structure sharp and distinct. To regulate the amount of light the **diaphragm** under the condenser must be brought into use. By moving a knob, *a*, on the side of the condenser (see Fig. 4) its shutters may be opened or closed, thus enlarging or reducing its central opening until the proper amount of light is obtained. In case of the microscopes having the wheel diaphragm in the condenser, it is revolved until the proper-sized hole is brought under the centre of the condenser.

Having made your study with the **low power,** you are next to turn on the **high power.** Select the point in the specimen to be viewed with the high power and place it in the field. Now throw up the tube of the microscope slightly with the coarse adjustment, and, taking hold of the high power, attached to the nose-piece, turn it into place under the tube. Now proceed to focus in the manner described above.

When working with the **high power, always keep the fine adjustment in motion,** turning it slightly to the right and then to the left. This brings the different planes of the specimen into view and a correct idea of its structure is obtained.

Recapitulation.

1. See that the field of the microscope is properly illuminated before the specimen is placed on the stage.
2. Adjust the amount of light to the character of the specimen.
3. Use the plane mirror when the high power is in use.
4. Use the coarse adjustment for approximate focussing only.
5. Use the fine adjustment for bringing the specimen into sharp focus. Always keep it in motion when the high power is in use. Never use it to do the work of the coarse adjustment.
6. In changing from the high to the low power, or from the low to the high power, always raise the tube of the microscope slightly.

Care of the Microscope.

The microscope being an instrument of precision, it must be handled with the greatest of care.

Do not remove or clean any of the optical parts of the instrument. If anything appears wrong, call the attention of one of the instructors to it and he will see that the difficulty is removed.

GENERAL TECHNIQUE.

Examination of Fresh Tissues.

Fresh tissues are to be examined in some **indifferent fluid,** that is, one which will produce but little or no change in the elements.

The indifferent fluids commonly used are as follows:

Physiological Salt Solution: a ¾ per cent. solution in water of sodium chlorid. Commonly called **salt solution.**

Iodized Serum: a saturated solution of iodin in amniotic fluid.

Ranvier's Iodin Solution: a saturated solution of iodin in a 2 per cent. solution of potassium iodid.

In addition to the above, perfectly fresh animal fluids, such as the aqueous humor of the eye, serous fluids, amniotic fluid, etc., may be used.

Fixing, Hardening, and Preserving.

Fixing.—By the term **fixing** is meant the rapid killing of the tissues, so that their elements will retain the same form and structure that they had during life.

A perfect fixing agent should kill rapidly; it should not dehydrate, thus causing shrinkage; it should not render the tissues brittle; and it should not interfere with the subsequent staining.

Numerous chemicals, in solution, are used for fixing, but at the present time we have no absolutely perfect one. Some cause shrinkage of certain elements, others swelling, etc. By combinations of various chemicals the faults of one may be partially overcome by the defects of others, so that some of the mixtures employed are nearly perfect.

The general rule to be observed in the use of fixing solutions is that the **pieces of the tissue must be small,** about 1 to 2 c.c. in

size, and that the amount of the solution be large, **100 times the bulk of the specimen.**

After fixation, which requires from 1 to 48 hours, the specimens are to be thoroughly washed in running water for from 12 to 24 hours, the time depending upon the fixative used and the size and the density of the specimen. After washing, the specimens are to be hardened in graded alcohols. (See Hardening.)

Osmic Acid is one of the best fixatives, but, unfortunately, it has but little penetrating power, so that it can only be used on exceedingly small bits of tissue. It is usually employed in 1 per cent. aqueous solutions and is allowed to act for 24 hours. Then wash the specimen in water and harden in 97 per cent. alcohol.

Osmic acid also stains fat black, and is used for staining tissues containing it.

Alcohol is the most commonly used fixing agent, though not a good one. Its use should be restricted to hardening and preserving.

Mercuric Chlorid is used in saturated aqueous solution, or, what is better, a saturated solution in ¾ per cent. salt solution. The pieces of tissue should be small, as it has but slight penetrating power. It should be allowed to act for not more than 24 hours; then the specimen is washed thoroughly in water; then placed in 80 per cent. alcohol tinged with tincture of iodin, the latter being added in small quantities until the alcohol ceases to become decolorized; then transfer to 97 per cent. alcohol.

Flemming's Fluid.—This is used in two strengths and is the best fixative for nuclear structures, but not for cytoplasm:

No. 1, or Weak Solution.

Osmic acid, 1 per cent. aqueous solution	8 c.c.
Chromic acid, 1 per cent. aqueous solution	25 c.c.
Hydric acetate, glacial, 1 per cent. aqueous solution	10 c.c.
Distilled water	55 c.c.

No. 2, or Strong Solution.

Osmic acid, 1 per cent. aqueous solution..... 10 c.c.
Chromic acid, 1 per cent. aqueous solution.... 25 c.c.
Hydric acetate, glacial, 1 per cent. aqueous solution..... 10 c.c.
Distilled water..... 55 c.c.

These solutions must be made fresh each time. Very small pieces of tissue are placed in the fluid for 24 hours; then washed in running water for at least 12 hours; then hardened in graded alcohols. (See Hardening.)

Zenker's Fluid.

Potassium dichromate..... 2.5 gms.
Sodium sulphate..... 1.0 gm.
Mercuric chlorid..... 5.0 gms
Hydric acetate..... 5 c.c.
Water ..... 100 c.c.

This fluid must be made fresh each time, or the salts may be dissolved in the water and kept as the stock fluid, to each 100 c.c. of which 5 c.c. of hydric acetate are added at the time of use.

Tissues should be fixed in this fluid for 24 hours, then treated in the same manner as those fixed in mercuric chlorid.

Müller's Fluid.

Potassium dichromate..... 2.5 gms.
Sodium sulphate..... 1.0 gm.
Water ..... 100 c.c.

For fixing, small pieces of tissue are placed in this fluid, in the dark, for 24 to 48 hours; then washed thoroughly in water and hardened in graded alcohols.

This fluid is used extensively for fixing and hardening the central nervous system. For this purpose pieces are placed in a large quantity of this fluid, in the dark, for three weeks to a month, the fluid being changed daily for the first week, then once

a week until the hardening is complete. They are then washed in running water and preserved in 88 per cent. alcohol.

Formalin is a 40 per cent. solution of the gas formaldehyde (HCOH) in water. It is used in 2½ to 10 per cent. solutions in water, and is both a fixing and hardening agent.

For fixing, pieces of tissue are placed in the fluid for 12 to 24 hours, then washed well in water, and preserved in 80 per cent. alcohol. For hardening, the tissues are allowed to remain in the fluid for 24 to 48 hours, then transferred to 80 per cent. alcohol.

Formalin is now used in combination with many of the other fixing agents, the most common being a modified Müller's fluid.

Formalin-Müller's Fluid (Orth's Fluid):

Potassium dichromate	2.5 gms.
Sodium sulphate	1.0 gm.
Formalin, 2½ per cent. aqueous solution	100 c.c.

This fluid should be made fresh each time, or the salts may be made up in a solution of double strength and at the time of using diluted with an equal volume of 5 per cent. formalin.

This fluid acts in a similar manner to the ordinary Müller's fluid, but more quickly, and has more penetrating power, so the pieces of tissue may be larger. It is to be used in the same manner as Müller's fluid.

Many other fixing solutions and methods of fixation are used for special cases; these will be considered under the special tissues and organs.

Hardening and Preserving.

All of the fixing solutions are also hardening agents, but for this purpose they must be allowed to act for a longer time, often to the detriment of the organs.

Alcohol.—Alcohol is the universal hardening and preserving agent. After the proper fixation of the specimens they must be thoroughly washed in running water; this requires in some instances one or more days, the size and density of specimen being a factor—usually 12 hours is sufficient. The specimens are then carried through graded alcohols, commencing with 60 per cent.

and changing each 12 hours, and increasing its strength 10 per cent. until 97 per cent. is reached.

For preserving fixed and hardened specimens 80 per cent. alcohol is to be used.

Maceration and Dissociation.

For the purpose of obtaining a correct idea of the form of the tissue elements it is necessary to **isolate** them. In many instances simple **teasing** of the fresh tissue with the points of the teasing needles is sufficient. In other cases, the cement substance uniting the elements being dense and strong, it is necessary to submit the tissue to the action of some chemical solution that will soften it. Such a solution is known as a **Macerating Fluid.**

The most common macerating fluid used is as follows:

Ranvier's One-third Alcohol.—Alcohol, 90 per cent., 1 part; water, 2 parts. This is an excellent macerating fluid for the epithelia. Small pieces of the fresh tissue are placed in this for 24 hours; then gently shaking in a test-tube, or teasing with needles on a slide, will isolate the elements. Except for a few special cases, this fluid may be used for all macerations.

Embedding.

In order to obtain thin sections of organs or tissues for microscopic examination, and at the same time hold all the elements in position during the subsequent manipulations, it is necessary to embed them.

For this purpose various embedding masses are employed, but at the present time they have practically been reduced to two, **paraffin** and **celloidin.** For our especial uses celloidin is employed.

Celloidin is a pure pyroxylin, free from foreign constituents, and it makes an opalescent solution free from sediment. It is manufactured by Schering & Co., of Berlin, and comes in the form of horny shreds.

For use a 5 per cent. solution of these shreds is made in a mixture of equal parts of alcohol and ether. This is the **strong celloidin,** and is to be diluted with alcohol and ether for making the other solutions.

The specimens to be embedded are first placed in 97 per cent. alcohol for a few hours; then in alcohol and ether for at least 12 hours; then in dilute celloidin (strong celloidin diluted with an equal volume of alcohol and ether) for 12 hours; then in strong celloidin until the specimens become **thoroughly impregnated.** This varies, in some cases 12 hours being enough, while in others 3 to 4 days will be required. Porous tissues, like the lung, require a short time; dense tissues, like the liver or kidney, a greater length of time. When the specimens have become thoroughly impregnated they are to be treated by one of the following methods:

(a) If the specimen be a piece of liver or similar dense organ, it is to be cut into rectangular-shaped blocks, then washed thoroughly in the thick celloidin and placed on the surface of a wooden block, or, what is better, a block of vulcanized fibre, pressed down, and allowed to stand in the air for 5 to 10 minutes, and then immersed in 80 per cent. alcohol to coagulate the celloidin; after 24 hours the block is ready for cutting sections.

(b) In case of small, thin, or irregular-shaped specimens, they are to be embedded in a paper box. The box is made by folding a rectangular piece of paper over the proper-sized face of a wooden block, turning down the edges of the paper at opposite ends to hold the box in shape.

The specimen is removed from the strong celloidin, placed in the centre of the box, which is filled with the celloidin. As soon as a strong film has formed on the surface of the celloidin, the box and its contents are immersed in 80 per cent. alcohol and allowed to remain for 12 hours. The paper is now removed from the block, and the specimen will be found embedded in a firm block of coagulated celloidin. This block is to be trimmed up into a rectangular shape, leaving considerable of a margin ($\frac{1}{16}$ of an inch) of celloidin around the specimen.

This block is now to be mounted on a block of vulcanite, as follows: Introduce a teasing needle into the celloidin block, immerse the block in a strong solution of celloidin, moving it about so as to thoroughly wash all of its sides. Then remove it and place it on a vulcanite block; press it down, and, after allowing it to stand in the air for 5 to 10 minutes, immerse the block in 80

per cent. alcohol. After 12 hours it will be ready for cutting sections.

SECTION-CUTTING.

Section-cutting is now done with an instrument called a **microtome**. The forms of microtomes are numerous, but all of the

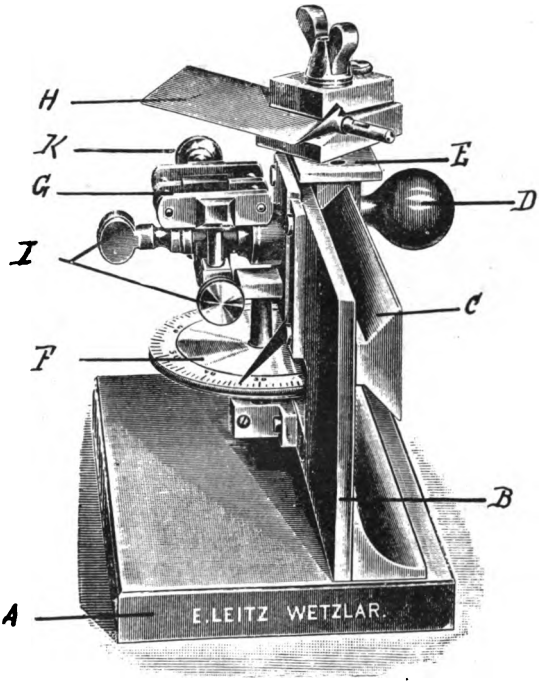


FIG. 7.—SMALL SLIDING MICROTOME.
(For explanation see text.)

more recent ones are constructed on the principle of the sliding microtome.

One of the smaller sliding microtomes is shown in Fig. 7. It consists of a heavy iron base, *A*, to which is firmly attached the perpendicular plate *B*. To the right side of the plate *B* is attached the runway, *C*, for the knife carrier *E*, which is moved by the knob *D*. The vulcanite block of the mounted specimen is

placed in the specimen clamp *G*, and fastened firmly by the screw *K*. The specimen clamp *G* is on two adjustable axes at right angles to each other, which permits of the surface of the specimen being adjusted to the proper plane for cutting. These axes are fixed by the screws *I*. The specimen clamp is moved in a vertical direction by a micrometer screw. The large circular plate *F* attached to it has its circumference graduated, each degree of which represents a vertical movement of the screw equal to 0.005 mm.

The knife *H* is clamped to the knife carrier *E*, and set at such an angle that it will pass through the specimen from heel to point.

For cutting sections with this instrument, the vulcanite is fastened in the specimen clamp. The knife is set at the proper angle and its surface flooded with 80 per cent. alcohol by means of a large camel's-hair brush. The circular plate *F* is turned sufficiently to bring the surface of the specimen some distance above the edge of the knife. Now, taking hold of the knob *D*, the knife is drawn quickly through the specimen, leaving a smooth surface. The knife is now pushed beyond the specimen; the circular plate, *F*, is turned so as to raise the surface of the specimen enough to give the required thickness of section. This should usually be $\frac{15}{1000}$ of a millimetre, and to give this thickness the circular plate should be moved through three degrees of its graduated circumference. The cut section is removed from the knife with a camel's-hair brush and placed in 80 per cent. alcohol for further manipulation. This process is repeated until the required number of sections have been cut. During the cutting of sections the runway of the knife carrier should be freely oiled and the surface of the knife flooded with 80 per cent. alcohol.

One of the newest forms of automatic sliding microtomes is shown in Fig. 8.

The base, *A*, is of metal, and from it rises the upright plate *B*, on one side of which the slides for the knife carrier (not shown in the figure) are attached. The knife carrier, *C*, shows the knife, *D*, clamped to its upper surface. The object clamp, *E*, is attached to the clamp carrier, *K*.

The knife carrier *C* moves on horizontal slides and is manipulated by the chain *H*, carried around the toothed wheel *G*, which is revolved by the handle *F*.

The object clamp *E* is raised vertically by the micrometer screw, to which is attached the toothed disc *I*. Each tooth of the disc equals an elevation of 0.0025 mm.

The automatic feed, *L*, is so arranged that when the knife carrier reaches the left end of the microtome it presses against the arm *M*, pushing it to the left, and causes a lever (not seen in the figure) to engage in the teeth of the disc *I*. This arm *M* is adjustable, so that it may cause the lever to move the disc any number of teeth from one to ten.

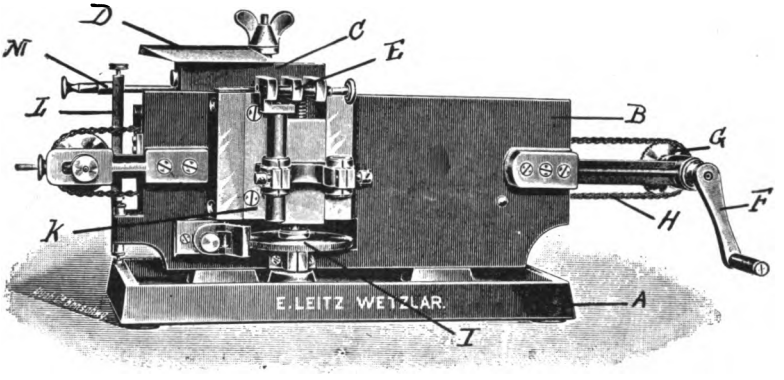


FIG. 8.—AUTOMATIC SLIDING MICROTOME
(For explanation see text.)

STAINING.

In order to bring out sharply the different tissue elements, the process of staining the sections is resorted to.

The stains used in our work are chiefly the **nuclear** and **plasma** ones—the former staining the nuclear structures only, the latter the cytoplasm of cells and intercellular substance. Selective stains are also used in certain cases. They stain certain elements more strongly than others, or select one element in preference.

Nuclear Stains.—For staining nuclei, solutions of carmin and hæmatoxylin are almost exclusively employed. The ones we shall employ are as follows:

Grenacher's Alum-Carmin:

Ammonia alum, 5 per cent. aqueous solution	100 c.c.
Carmin	1 gm.

Boil for 15 minutes; cool and filter. Add a crystal of thymol to prevent the growth of moulds. This solution stains nuclei of a purple red color and does not over-stain. The stain is rather slow in its action, requiring at least half an hour.

Delafield's Hæmatoxylin:

Hæmatoxylin crystals	1 gm.
Alcohol	6 c.c.
Saturated aqueous solution of ammonia alum	100 c.c.

Dissolve the hæmatoxylin in the alcohol and add to the alum solution. Allow the mixture to stand in the light for a week to ripen, when it will become a dark purple color; then filter. Then add 25 c.c. of glycerin and 25 c.c. of wood naphtha. After standing 2 to 3 days, filter. For use, dilute one-half with water. Stain sections for 2 to 5 minutes, wash in water. Nuclei stain bright bluish purple.

Gage's Hæmatoxylin:

Distilled water	200 c.c.
Ammonia alum	7.5 gms.
Chloral hydrate	4.0 gms.
Hæmatoxylin crystals	0.1 gm.

The alum is added to the water and boiled for 15 minutes to half an hour. This is to destroy any germs in the water or alum. After cooling, add to this solution the hæmatoxylin dissolved in the alcohol, and then the chloral hydrate. Allow the mixture to stand for a week to ripen, and then filter.

For staining, this solution may be used full strength or diluted one-half with water.

Heidenhain's Iron Hæmatoxylin.—This is a double process,

the sections being first mordanted in the iron solution and then stained in the hæmatoxylin.

The mordant is a 2½ per cent. aqueous solution of ammonium sulphate of iron. Sections are placed in this solution for 4 to 8 hours, then rinsed in water and placed in the staining solution, which is a 1 per cent. solution, in water, of hæmatoxylin, in which they are allowed to remain for 12 to 24 hours. They are then rinsed in water and placed in the iron solution for differentiation. The sections which are stained black are moved about in the iron solution, clouds of color being given off, until they become of a grayish tint; they are then washed thoroughly in several changes of water.

Nuclear structures stain a jet black, especially the centrosome; the cytoplasm grayish or may be clear. This is an excellent stain for mitotic figures.

It is now known that the active staining in hæmatoxylin solutions is **hæmatin**, and that it is formed by the action of the oxygen of the air coming in contact with the hæmatoxylin, converting it into hæmatin.

At the suggestion of Paul Mayer, **hæmatin** is now being used in place of hæmatoxylin, thus doing away with the "ripening" process of the old solutions.

Mayer's Hæmalum:

Hæmatin, 1 gm., is dissolved in 50 c.c. of alcohol; this is added to 1,000 c.c. of a 5 per cent. aqueous solution of ammonia alum. This stain may be used immediately; it stains quickly (3 minutes), never over-stains when diluted with water, and penetrates quickly.

We now use a staining solution which is a combination of Gage's and Mayer's formulæ. It is prepared as follows:

Aqueous solution, 5 per cent., of ammonia alum is boiled, or, what is better, placed in a steam sterilizer for one hour. When cool, 0.5 gm. of hæmatin dissolved in 50 c.c. of alcohol and 20 gms. of chloral hydrate are added. This solution is used in full strength. It stains in about three minutes.

Plasma Stains.—A plasma stain is one that colors all of the tissues except the nuclei of cells. The color varies with the differ-

ent elements, some taking up the stain more intensely than others, thus a somewhat selective action being obtained.

Eosin.—This is a coal-tar dye and is found in commerce under a variety of names. It stains tissues various shades of rose red. The eosin we employ is prepared as follows: A saturated aqueous solution of water-soluble eosin is precipitated by hydric chlorid and the precipitate brought on a filter, washed with water until the filtrate becomes tinged with the eosin. The precipitate is then allowed to become thoroughly dry; then pulverized and made up into solution in the proportion of 1 gm. of eosin to 1,500 c.c. of 97 per cent. alcohol.

MOUNTING.

Specimens, after having been stained, are mounted on glass slides for study and preservation.

The **slide** is a slip of plate glass, 1 by 3 inches, with the edges smoothed off. The slides should be about one-sixteenth of an inch thick and free from all blemishes. For covering the specimen, **cover-glasses,** very thin pieces of glass, are used; they may be either square or circular in form, the size being what is known as the ¾ inch.

Before use both slides and cover-glasses must be thoroughly cleaned. This is done by wiping them carefully with a clean, soft linen cloth. Sometimes the slides and covers when received from the dealers are covered with an oily material. In such cases they should be wiped with a cloth moistened with alcohol, and then polished with a dry one.

Media.—The common media used are **Canada balsam** and **glycerin.**

Canada Balsam.—The ordinary balsam of commerce is not to be used for mounting, but must undergo special treatment.

Commercial balsam is heated in a shallow dish, placed on a sand-bath, until all volatile matter is driven off, and it becomes, when cold, hard and brittle. Care must be taken not to use much heat or the balsam will become dark in color.

This hard balsam is dissolved in xylol, benzol, or oil of cedarwood for use. For our purpose we use oil of cedarwood as the solvent.

Glycerin.—Perfectly pure, clear glycerin is to be used. In double stains it is necessary to tinge the glycerin with a solution of eosin, or the eosin stain will be withdrawn from the specimen.

Specimens that are to be mounted in glycerin may be placed directly in it after washing out the surplus nuclear stain.

Balsam.—Specimens to be mounted in balsam have to be carried through several preliminary stages. After staining the nuclei and washing they are put into 97 per cent. alcohol for partial dehydration; then in the **eosin-alcohol,** which completes the dehydration and at the same time stains the specimen.

As alcohol and balsam do not mix, it is necessary to carry the specimen through some medium that is miscible both with alcohol and balsam. This is known as clearing the specimen. One of the essential oils is used for the purpose. Many oils—oil of cedar, oil of cloves, oil of bergamot, etc.—are used. In cases where the specimens have been embedded in celloidin an oil must be used that does not dissolve it. All of the above-mentioned oils have more or less action on the celloidin, consequently they must not be used. There are a few oils that do not attack the celloidin, and one is the **oil of origanum Cretici.**

For clearing, the specimen is taken from the eosin-alcohol and floated on the surface of the oil of origanum, contained in a flat, shallow dish. The alcohol in the specimen is gradually replaced by the oil; it becomes clear, and finally, when all of the alcohol has been removed, the specimen becomes transparent and sinks to the bottom of the dish.

The specimen is now removed from the oil by drawing up on a **lifter** with the point of a **teasing needle,** and placed on the centre of a slide, all folds being removed. The surplus oil is removed with filter paper. A piece of filter paper is folded **four** times, placed on the specimen, and pressed down gently; the paper absorbs all the oil, leaving the specimen flattened out on the slide. A drop of balsam is now placed on the specimen and the cover-glass put on.

One edge of the cover-glass is taken in the points of the forceps; the opposite edge is brought in contact with the balsam, gradually lowered to the horizontal position, and then allowed to settle by its own weight.

The edges of the cover-glass of specimens mounted in glycerin are to be cemented to the slide. All traces of glycerin must be removed or the cement will not adhere to the glass. This is accomplished by washing out a camel's-hair brush in strong alcohol, absorbing the greater part of the alcohol by wiping the brush on filter paper until it is only moist, and then wiping around the edges of the cover-glass with the brush until all the glycerin is removed from the slide. After each wipe the brush is to be washed out in alcohol and passed over the filter paper as above.

For cementing the cover-glasses the following shellac varnish is used: Shellac is dissolved in strong alcohol, so as to obtain a thick solution, and 20 drops of castor oil added to each cubic centimetre of the shellac solution. This is painted around the edges of the cover-glass with a small camel's-hair brush, so that the line of cement shall lap slightly on the surface of the cover.

Specimens mounted in balsam must not be cemented. After being mounted for a month or six weeks, the balsam will have hardened sufficiently to allow of the surplus being scraped from the slide.

Staining Double and Mounting in Glycerin.

1. Place the specimen, from water, in the hæmatoxylin stain for from *3* to *5* minutes.

2. Remove the specimen from the stain and wash thoroughly in water.

3. Place the specimen in the centre of a slide, taking care to remove all folds and see that it lies perfectly flat.

4. Remove the excess of water by absorbing it with bits of filter paper.

5. Place a drop of **glycerin** or **eosin-glycerin** on the specimen and put on a cover-glass.

6. Clean the slide of the excess of glycerin.

7. Cement the cover-glass to the slide.

Staining Double and Mounting in Balsam.

1. Place the specimen, from water, in the hæmatoxylin stain for from *3* to *5* minutes.

2. Remove the specimen from the stain and wash thoroughly in water.

3. Place the specimen in *97* **per cent. alcohol** for *3* minutes for partial dehydration.

4. Transfer the specimen to **eosin-alcohol,** to complete the dehydration and staining, *3* minutes.

5. Float the specimen on oil of origanum, and as soon as it has become transparent transfer to a slide with the lifter.

6. Smooth out the specimen and remove the surplus oil with filter paper.

7. Put on a drop of Canada balsam and apply the cover-glass.

Do not cement the cover-glasses of specimens mounted in balsam.

PART SECOND

The Cell, the Tissues, Lymphatic Organs, Gastro-Intestinal Canal, Liver, Kidney, and Respiratory Organs.

THE CELL.

The word cell is derived from the Latin "cella," a little cavity or space. This was what the earlier investigators believed it to be, but we now know the cell to be a solid body.

It was the botanists who first gave us the idea of the structure of living matter. At about the end of the seventeenth century Malpighi and Grew, with their crude instruments, demonstrated that plant tissue was made up of small cavities filled with fluid cells. As their investigations progressed they found that in addition to the fluid a granular mass was present and that it contained a roundish, darker spot; this spot is now known as the nucleus.

At a slightly later period the anatomists investigated the structure of animal tissue, and Purkinje, Valentin, Müller, and Henle (1830-1840) demonstrated that its elements were like those of plant tissue. Schwann in 1830 announced as the results of his examinations that plant and animal tissue were composed of similar elements—cells—and defined a cell as "a small vesicle, with firm walls, enclosing fluid contents."

Further investigation of the plant cell showed that in many instances the cell was completely filled with a granular substance; this was named **protoplasm** by Mohl.

As the investigation of the animal cell proceeded numerous facts as to its structure were brought out. It was found that in most cases the cell wall was absent, and that their structure was identical with the protoplasm of Mohl. Remak applied the name "protoplasmic bodies" to them.

Max Schultze in 1860 announced his protoplasmic theory of the animal cell. He called attention to the fact that the cell wall was not an essential part of cells; that plant protoplasm was generally surrounded by a firm wall, but at times it lost it and then its characteristics were the same as animal protoplasm. He also demon-

strated that the element was not a cell or space filled with fluid, but a formed material. He, however, retained the term cell and defined it "as a little mass of protoplasm endowed with the attributes of life." We still retain the term cell on account of its long usage.

Further investigation of the cell showed a more and more complicated structure. The dark mass, now known as nucleus, in the protoplasm was found to be a very important part; the remainder of the protoplasm, the cell body, was found to be different from the nucleus. As the results of these investigations a revision of

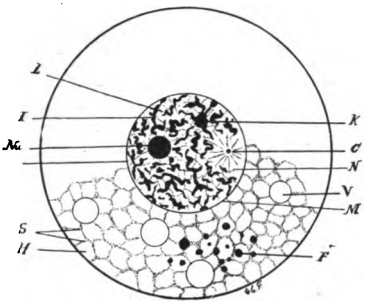


FIG. 9.—DIAGRAM OF A CELL.

N, nucleus; *C*, centrosome; *Nu*, nucleolus; *K*, karyosome or net-knot; *I*, intranuclear network (chromatin); *L*, nin; *M*, nuclear membrane; *S*, cytoreticulum or spongioplasm; *H*, hyaloplasm; *V*, vacuoles; *F*, foreign bodies.

Max Schultze's definition became necessary and the following was formulated:

"A cell is a little mass of protoplasm which contains in its interior a specially formed mass, the nucleus."

During the last few years a vast amount of information as regards the structure of the cell has accumulated; this is especially the case as regards the function of the nucleus and the part it takes in the process of cell division.

STRUCTURE OF THE CELL.

The cell is one of the elementary forms of organized substance of plants and animals. It is irreducible into more simple parts,

except by mechanical or chemical means. It therefore is the **Histologic Element.**

1. **Cell Body,** Protoplasm,[1] or Cytoplasm.
   - *a.* Spongioplasm or cytoreticulum.
   - *b.* Hyaloplasm.
   - *c.* Microsomes.
2. **Nucleus,** or Karyoplasm.
   - *a.* Nuclear membrane.
   - *b.* Intranuclear network.
     - *a.* Chromatin.
     - *b.* Linin.
   - *c.* Karolymph, or nuclear sap.
   - *d.* Nucleoli.
3. **Centrosome.**
4. **Cell membrane.**

Cytoplasm (Cell Body, Protoplasm).—Cytoplasm, under the ordinary powers of the microscope, appears homogeneous or in most cases granular. Analysis with high powers and special methods of preparation shows that it is composed of two substances, **spongioplasm** or **cytoreticulum** and **hyaloplasm.**

Spongioplasm, or **cytoreticulum,** forms an irregular network arranged somewhat after the manner of a sponge. It is believed to be homogeneous in structure.

Hyaloplasm is fluid or semi-fluid in nature and fills the spaces of the cytoreticulum. Embedded in it are minute granules, the **microsomes.** These are not distributed equally; usually the peripheral portions contain less than the central. They may be numerous and coarse, giving the cytoplasm a dark appearance; or fine and less numerous, when it appears finer and more clear. In addition to the microsomes the hyaloplasm may contain granules of pigment, fat, foreign bodies, and in some instances clear, spherical-shaped cavities—**vacuoles.**

There are two theories as to the minute structure of cytoplasm —the **foam or emulsion theory** of Butschli and the **reticular theory** of other investigators. The reticular theory has the most supporters.

Nucleus, or **karyoplasm,** is the important part of the cell. It is generally embedded in the cytoplasm. In a few cases it projects above the surface. Its elements stain deeply with certain dyes.

[1] The term protoplasm is now used as a morphological one entirely.

It may be spherical, oval, rod-like, or irregular in shape. Its size is in proportion to the size of the cell. Every cell, with a few exceptions, has one nucleus, sometimes two or more. The exceptions are the red blood cells and respiratory epithelium.

Nuclear Membrane surrounds the nucleus and separates the nuclear contents from the cytoplasm. It is divided into two layers, **inner,** which stains—**chromatic**; and **outer,** which does not stain—**achromatic.**

The **nuclear contents** is composed of two substances, the **intranuclear network,** or **reticulum,** a formed material, and the **nuclear sap,** or **karyolymph,** which is believed to be fluid.

The **intranuclear network** is composed of threadlike structures, the **chromatin** and the **linin.** The chromatin stains, the linin is unstainable. The **chromatin** is in the form of irregular anastomosing threads, which are supported by the linin. The chromatin threads vary in thickness; in some nuclei they are coarse, in others thin, and in a few instances they appear as round or irregular-shaped granules. The **linin** occurs in thin, unstainable threads.

The **nucleoli** are of two kinds—**true nucleoli,** or **plasmasomes,** and **false nucleoli,** or **karyosomes.** The **true nucleoli** are spherical in shape; stain deeply; they lie free in the nuclear sap or may be attached to the intranuclear threads. The **karyosomes,** or false nuclei, are thickened points of the intranuclear network.

Centrosome.—A minute, spherical-shaped body found sometimes within the nucleus and sometimes in the cytoplasm near the nucleus. It is surrounded by a zone of radiating, unstainable fibrils, the attraction sphere or archoplasm. It is believed to be a special part having direct control over the process of cell division.

Cell Membrane.—This is now considered an unimportant part of the animal cell, only being present in but a few instances, in the ovum, where it is well developed, and in fat cells.

Shape of Cells.—Cells may be oval or spherical, the shape of all young cells; flat, cylindrical, or columnar, as in the epithelial tissues; discoid, the shape of the red blood cells; irregular, nerve and connective-tissue cells. Pressure modifies the shape of cells; this is well seen in cases where cells are stratified.

Vital Properties of Cells.—The vital properties of cells consist of the phenomena of **movement, irritability,** and **reproduction.**

Movement occurs in three forms: **protoplasmic, amœboid,** and **ciliary.**

Protoplasmic movement is a slow process and difficult of observation. All animal cells possess it to a greater or less degree. It may be detected by changes in the position of the nucleus, movements of the microsomes, and change in shape of the cytoplasm.

Amœboid movement resembles that of the unicellular organism, the amœba. It is well marked in some of the white blood cells, the leucocytes, the lymph cells, and the wandering connective-tissue cells. It will be considered more in detail when we study the blood cells.

Ciliary movement is the movement of hair-like processes, the cilia, of certain epithelial cells. (See Epithelium.)

Irritability is the power cells have of responding to external stimuli.

Reproduction.—All cells are reproduced from pre-existing cells. The ovum is the starting point in the animal, and all of the multitude of cells of the various tissues and organs are derived from it. A cell (mother cell) divides, forming two cells (daughter cells); these daughter cells again divide, forming four, and so on.

This process is known as **cell division,** and two forms are now recognized: **indirect cell division,** or **karyokinesis,** or **karyomitosis,** or **mitosis,** and **direct cell division,** or **amitosis.**

Mitosis, or indirect cell division, is the process by which most of the cells in the body divide. The intranuclear network undergoes a series of complicated changes; the nucleus divides, forming **daughter nuclei,** which is followed by division of the cell body, forming **daughter cells.**

The process is divided into **four stages: prophases,** a preparation of the nucleus for division; **metaphases,** commencement of the division of the nucleus; **anaphases,** distribution of the nuclear material; **telophases,** complete division of the nucleus and cell body and return of the daughter nuclei to the resting stage.

Prophases.—The resting nucleus (Fig. 10 A), in which the in-

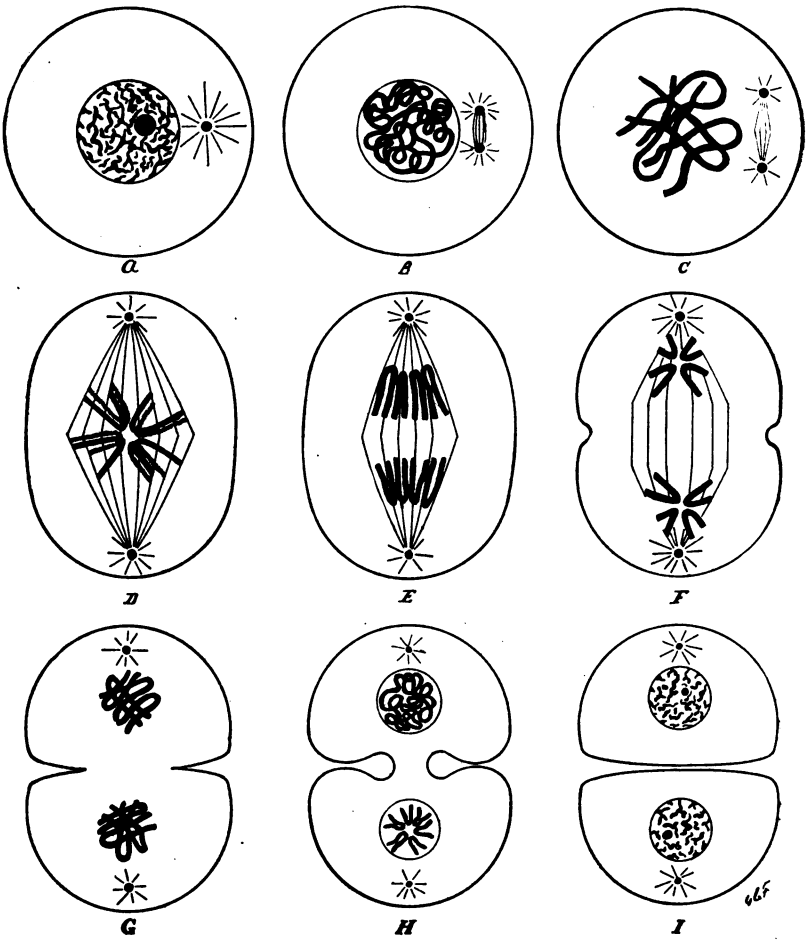


FIG. 10.—SCHEMATIC REPRESENTATION OF MITOSIS, AFTER BÖHM AND VON DAVIDOFF.

A, resting nucleus; *B*, coarse skein; *C*, wreath breaking up into V-shaped segments; *D*, monaster, chromosomes dividing; *E*, distribution of chromosomes; *F*, diaster; *G*, cell body dividing—formation of daughter nuclei; *H*, further division of cell body—daughter nuclei, one showing monaster, the other coarse skein; *I*, complete division of cell body forming daughter cells—nuclei resting stage.

tranuclear network is of an irregular shape, enlarges. The **centrosome** which has been within the nucleus passes out into the cytoplasm (Fig. 10 A), and, dividing, forms two centrosomes—**daughter centrosomes**—which become separated from each other and lie near the nucleus (Fig. 10 B). Each daughter centrosome is surrounded by the radiating fibrils of the **archoplasm**, some fibrils of which, stretching between the centrosomes, form the **achromatic spindle** (Fig. 10 B). The irregular-shaped intranuclear network becomes transformed into a fine, convoluted thread, which is probably continuous. The thread next breaks up into several short threads, which thicken, and the convolutions become reduced in number (Fig. 10 B). At this stage the chromatin stains intensely and resists the action of decolorizing agents. The chromatin becomes arranged in a wreath-like form, which breaks up into a series of V-shaped masses, the **chromosomes** (Fig. 10 C). The nuclear membrane and nucleolus disappear. The nucleolus is believed by some to pass into the cytoplasm and there degenerate. The chromosomes are always of an equal number, varying in each species of animal, being from two to thirty-six. In man they number sixteen.

The centrosomes pass to opposite ends of the nucleus—poles of the nucleus (Fig. 10 D)—and some of the threads of the archoplasm, stretching between them, in the long axis, form the **achromatic spindle** (Fig. 10 D). The chromosomes gather around the axis of the nucleus, at its equator—the **equatorial plane**—and grow shorter and thicker. Their closed ends are directed toward the centre and they form the **monaster** (Fig. 10 D).

Metaphases.—In this stage the actual division of the nucleus commences. The chromosomes become divided, longitudinally, into halves—the daughter chromosomes. These daughter chromosomes remain gathered around the axis at the equatorial plane.

Anaphases.—In this stage the equal distribution of the nuclear material takes place. The chromosomes separate into two equal groups. Each group passes to one of the poles of the nucleus Fig. 10 E, F), and, collecting there, in a manner similar to the monaster stage, they form the **diaster** (Fig. 10 F). Toward the end of this stage a constriction of the cytoplasm begins to take place at the equatorial plane.

Telophases.—The constriction of the cytoplasm deepens until the original cell becomes divided into two halves—daughter cells—each daughter cell receiving one of the nuclei and centrosomes (Fig. 10 G, H, I). The nuclear membrane is reformed and surrounds the nucleus, and the nucleolus reappears. The daughter nuclei now return to the resting stage by a reversal of the above-described process.

Amitosis, or Direct Cell Division.—In this form the intranuclear network does not undergo the complicated process of mitosis. The process is believed to be confined to lymph cells and leucocytes.

In this form of division the nucleus becomes constricted; the constriction deepening cuts the nucleus into halves. These gradually draw away from each other, and at times complete division is delayed, the nuclei being connected by threads of the nuclear material. Division of the cytoplasm by a constriction between the daughter nuclei, which deepening divides the cell into halves. Sometimes the division of the cytoplasm is also delayed while that of the nuclei goes on. As a result of this multinucleated cells are formed.

Practical Study.

Red Blood Cells of the Frog.—A frog is killed by cutting across the spinal cord just behind its ears. The thoracic cavity is opened and the heart exposed. The apex of the heart is cut off with scissors and 3 to 5 drops of the blood are allowed to drop into 100 c.c. of Hayem's[1] fluid contained in a tall, cylindrical-shaped vessel. The cylinder is then shaken so as to disseminate the cells through the fluid, and allowed to stand for 12 hours to allow the cells to settle. The fluid is then carefully decanted and water added; after the cells have settled this is poured off and 80 per cent. alcohol tinged with iodin added. After standing for 12 hours the alcohol is decanted and 25 c.c. of alum-carmin added for staining the nuclei. This is allowed to act for 24 hours; then the cells are washed with water by decantation, and finally preserved in glycerin tinged with a saturated aqueous solution of picric acid.

[1] *Hayem's Fluid.*—Sodium chlorid, 1 gm.; sodium sulphate, 5 gms.; mercuric chlorid, 0.5 gm.; distilled water, 100 c.c.

A drop of the glycerin containing the cells is placed on a slide, covered with a cover-glass, and placed on the stage of the microscope.

Low Power.—Find a collection of the cells; place it in the **centre of the field** of the microscope by slowly moving the slide; change to the

High Power.—Observe the shape of the cells. Note the **nucleus,** stained red, in the central part of the **cytoplasm,** or **cell body.** Also note that the nucleus is not homogeneous, but that by careful focussing a coarse network—**intranuclear network**—can be brought into view. Note the **cytoplasm,** or **cell body,** stained yellow, is slightly granular or in some cells reticular in appearance.

Make a drawing of a cell seen on the edge and on the flat.

Cells from the Bladder.—The bladder is removed from a recently killed animal and pinned out on a piece of sheet cork, with as little stretching as possible, the mucous membrane uppermost. The cork, with the specimen down, is floated on Müller's fluid diluted one-half with water or Ranvier's alcohol and allowed to remain for 24 hours. Then remove the cork and float it on water to wash out the **maceration fluid.** Remove the cork from the water and with a knife gently scrape the surface of the specimen, removing the cells. These are placed in alum-carmin for staining the nuclei, and after washing in water, as described under the first specimen, are preserved in glycerin tinged with picric acid.

Mount on a slide as described under the first specimen.

Low Power.—Proceed as with the first specimen.

High Power.—Observe that the cells are not all of the same shape or size. Note the groups of large surface cells arranged like a mosaic; the large isolated cells seen on the edge and on the flat; the smaller cells from the deeper layers. Also note the nucleus—some of the cells may have two—and the intranuclear network; the character of the cell body. Is it granular or clear? These cells show how pressure can modify the shape of cells.

Make a drawing of a large surface cell as seen on the flat, showing the details of the structure of the nucleus and cell body.

TISSUES.

A tissue is composed of **cells** and **intercellular substance.**

The proportion of cells and intercellular substance varies in the different tissues. In young tissue the cells predominate; in older tissue the intercellular substance is generally greater.

The physical condition of the intercellular substance also varies. In young tissue it is semi-fluid or gelatinous; in blood it is fluid; in older tissue it is dense; and in bone it is hard.

Origin of Tissues.

All tissues are derived from one cell, the ovum. In order that this cell may go on and develop it must be fertilized, the fertilization being produced by the male element, the spermatozoon.

After fertilization of the ovum it divides by mitosis into two cells, these into four, and so on. At first all the cells are similar, being spherical and without any special characteristics. These are the **undifferentiated cells.** As the process of multiplication goes on, a point is reached where certain cells begin to assume special characters—**differentiated** cells—and begin to arrange themselves in layers, the **germ layers.**

Layers of the Blastoderm.

The cells resulting from the division of the ovum having reached a certain point, differentiation begins and two kinds of cells are formed, the **clear cells** and the **granular cells.** The granular cells gather near the centre of the mass and are surrounded by the clear cells. The granular cells move to one side; the clear cells flatten out and line the interior of the membrane of the blastodermic vesicle.

All the cells divide by mitosis, the granular more rapidly than

the clear ones. The granular cells as they multiply spread out, at one point, on the inner surface of the clear ones; and finally three distinct layers of cells are formed, the **layers of the blastoderm.**

From these three layers all of the tissues of the body are developed, each layer giving origin to certain specific tissues.

From the **outer layer,** or **ectoderm,** are derived the epidermis of the skin, the epithelium lining the buccal cavity and salivary glands, the epithelium of the nasal tract, the lens of the eye and retina, the epithelium of the internal ear, and the elements of the central nervous system.

From the **middle layer,** or **mesoderm,** are derived the elements of the connective-tissue group: the blood and lymph cells; the epithelium of serous membranes; the epithelium of the uriniferous tubules and ureter; the epithelium of the generative organs; the endothelium of blood and lymph vessels; muscular tissue; spleen and lymph nodes.

From the **inner layer,** or **entoderm,** are derived the epithelium lining the intestinal tract; the epithelium of the respiratory tract; the epithelium of the bladder and urethra (in the male, the prostatic portion only); the liver; and the pancreas.

According to Minot the mesoderm becomes differentiated into three sublayers:

1. **Mesothelium,** which retains the character of epithelial cells and lines the cavities of the peritoneum, pleura, and pericardium. It also furnishes the epithelium of the genito-urinary organs, with the exception of the bladder and urethra.

2. **Mesenchyme,** which furnishes the elements of connective tissue, involuntary muscle, spleen, lymph nodes, bone marrow, and endothelium of blood and lymph vessels.

3. **Mesamœboid cells,** the blood and lymph cells.

Classification of Tissues.

The tissues are classified as follows:

1. Blood and lymph.
2. Connective tissue.
3. Muscular tissue.
4. Nervous tissue.

BLOOD.

Blood is a tissue in which the **intercellular substance** is fluid, the **blood plasma.** The formed elements are of three kinds—**red cells, white cells,** and **plaques.**

Red Cells.—In the human subject the red cells are biconcave discs without nuclei, the average size being 7.9 microns, or $\frac{1}{3200}$ of an inch, in diameter.

In thin layers under the microscope they appear homogeneous and are of a pale yellow color.

They are composed of a **stroma** and coloring matter, **hæmoglobin,** which is distributed through the stroma. The hæmoglobin may be dissolved out with water, which causes the cells to swell up and become spherical.

In fresh preparations, when seen under the microscope, the red cells run together in rows, or **rouleaux.** After a time, owing to the concentration of the plasma from loss of water by evaporation, the cells begin to change in form, becoming **crenated.** The white cells appear as bright, granular, spherical-shaped bodies, no nucleus being visible.

After the blood has been withdrawn from the blood vessels it soon **coagulates.** This is caused by the separation of the **fibrin** from the plasma, which coagulates in the form of a network of thin threads, and, enclosing the cellular elements, forms the **blood clot.** After standing the blood clot contracts and a fluid is forced out, which is **blood serum.**

The number of red cells average 5,000,000 in males and 4,500,000 in females to the cubic millimetre of blood.

White Cells.— The white cells are spherical-shaped, nucleated bodies with a granular cytoplasm. They vary in size, some being smaller, others larger than a red cell. They average 7,300 to a

cubic millimetre of blood, or one to from three hundred to five hundred red cells.

Classification.—The **white blood** cells may be grouped under four classes, as follows:

1. **Lymphocytes.** These are again subdivided into two groups:

(a) **Small Lymphocytes** have a diameter of 5 to 7 μ, being smaller than a red cell. They are spherical in shape, with a large, spherical-shaped nucleus which stains deeply. The cytoplasm is finely granular and surrounds the nucleus as a narrow zone.

(b) **Large Lymphocytes** have an average diameter of 7.5 μ. The nucleus is large, spherical in shape, and stains deeply. The cytoplasm is greater than in the former.

Lymphocytes form about 20 per cent. of the total number of the white cells.

2. **Large Mononuclear Leucocytes.** The large mononuclear leucocytes are about the size of a large lymphocyte. The nucleus is spherical in shape and stains faintly. The cytoplasm is large and finely granular. The granules stain with the basic anilin dyes. They form about 2 to 4 per cent. of the total number of white cells.

3. **Transitional Leucocytes.** The transitional leucocytes are about the same size as the mononuclear. The cytoplasm is large in amount, finely granular, the granules staining with the basic anilin dyes. The nucleus is about the same size as that in the mononuclear, but is curved or irregular in shape. This form of leucocyte is about 2 to 4 per cent. of the total number of white cells.

The transitional leucocytes are developed from the mononuclear and is one of the stages of development of the true or polynuclear leucocyte.

4. **Polynuclear Leucocytes.** This form of leucocyte is commonly called the true leucocyte. It is slightly larger than a red cell and has one or more nuclei. Sometimes the nuclei, which are formed by fragmentation of the original nucleus, remain connected by thin bands of nuclear material, giving at first glance the appearance of an irregular-shaped nucleus. The cytoplasm, which is comparatively large, is granular, the granules varying in size in different cells. On account of the reaction of these

granules to the anilin dyes, the polynuclear leucocytes are divided into two groups, the *(a)* **neutrophile,** *(b)* **eosinophile.**

(a) **Neutrophile Polynuclear Leucocytes.** The cytoplasm of this form is rather coarsely granular, the granules staining with the *neutral* anilin dyes. They are the most numerous of all the forms of leucocytes, amounting to about 70 per cent. of the total number of white cells. They possess the power of *amœboid movement.*

(b) **Eosinophile Polynuclear Leucocytes.** The cytoplasm of this form of leucocyte is coarsely granular, the granules staining an intense red with the *acid* anilin dyes—eosin. They are the most actively amœboid of all the leucocytes. Normally they are but few in number, about ½ to 4 per cent. of the total number of white cells.

Basophile leucocytes have been described as occurring in normal blood.

The **amœboid movement** of the leucocytes consists of alternate extension and contraction of portions of the cytoplasm. These extensions, or **pseudopodia,** are given off at one or several points; some continue to extend, others are withdrawn; at times one becomes more active, and, becoming fixed, gradually draws the remainder of the cytoplasm after it. By this means the leucocyte will travel over considerable distance. It is by means of this amœboid movement that the leucocytes pass through the walls of capillary blood vessels and wander about in the spaces of the tissues or between other cells.

An important function of the true leucocyte is its absorption of tissue particles and foreign bodies. In the first instance it plays an important part in tissue disintegration, and in the second it removes particles from the body which might become harmful It probably, also, plays an important part in the process of assimilation.

Origin of Blood Cells.

Red Blood Cells.—Early in the development of the fœtus masses of cells of mesenchymic origin appear in the *area vasculosa* of the blastoderm. These collections of cells are known as the **angioblastic cells** (Schafer), or the **blood islands of Pander.**

It is from these cells that the fœtal red blood cells are developed. They are nucleated cells and lose their nuclei late in fœtal life. The liver has been supposed to form fœtal red blood cells, but now it is quite definitely settled that it is only an organ where the cells divide by mitosis.

Late in fœtal life and after birth the red blood cells are derived chiefly from a special cell found in the red marrow of bones—the **erythroblasts**. It is also stated that the spleen gives origin to red cells.

White Blood Cells.—The lymphocytes are developed from the lymphatic tissue found in the lymph nodes and nodules. It is from special parts of this tissue that they take their origin, the *germ centres* of Flemming. From these centres they pass into the lymph circulation and from this into the blood.

They are also developed in the spleen, and probably, also, in the bone marrow.

Practical Study.

Fresh Human Blood.—Prepare the specimen as follows: Wind a piece of cord around the index finger of the left hand, in a spiral manner, commencing at the base of the finger. This produces a congestion of the end of the finger. Puncture the skin, at the side of the nail, with the needle. Transfer a drop of blood to the centre of a slide and put on a cover-glass at once.

High Power.—Observe the rows of red blood cells on the edge, forming the **"rouleaux"**; the single red cells seen on the flat; the **crenated** red cells; and the white cells.

Make a drawing of a single red cell as seen on the flat; of a "rouleau"; of a white cell; and of a crenated cell.

Place a drop of hydric acetate in contact with one edge of the cover-glass, and a small bit of filter paper at the opposite side (it may be necessary to loosen the cover-glass with the point of a teasing needle). Examine with the high power and note the action of the hydric acetate on the red cells.

This specimen is not to be preserved.

Section of Blood Clot Showing Fibrin.—For the demonstration of fibrin one of the following methods may be used: A medium-sized drop of blood is placed on a slide and covered; in a few

moments the blood will have coagulated; then the cover-glass is raised with the forceps, and the cells washed out of the clot by allowing water to drop on it from a pipette. The cover-glass is now inverted and placed on a drop of **eosin-glycerin** on a slide. Then place on the stage of the microscope and examine with the **high power.**

Or sections may be made from a blood clot occurring in some tissue. A section is mounted in **eosin-glycerin** and the study made with the **high power.**

Note the network of the fibrin fibres, and make a drawing of them.

Hæmoglobin Crystals from the Blood of a Rat.—A small drop of the blood of a rat is mixed on a slide with an equal quantity of water, a cover-glass is put on, and the specimen examined with the

High Power.—The water dissolves out the hæmoglobin from the red cells, which swell up and practically disappear. After a few moments prismatic crystals of hæmoglobin will begin to appear near the edge of the cover-glass; these will gradually grow in size, and after 5 to 10 minutes crystals of large size may be seen. Make a drawing of several crystals.

This specimen may be cemented, when the crystals will be preserved for some time.

Permanent preparations of human blood may be made by allowing a drop of fresh blood to fall into 100 c.c. of Hayem's fluid (see page 38). After 24 hours the fluid is decanted from the cells; they are washed in water by decantation, and finally preserved in glycerin.

Spread of Human Blood, Nuclei of White Cells Stained.—A drop of blood is obtained from the finger as described under the first specimen. Have two clean slides ready; one is placed on the table and a drop of blood taken up on the end of the other from the finger. Hold the slide perpendicular and bring the drop of blood in contact with one end of the surface of the slide on the table. Draw the perpendicular slide, quickly and with slight pressure, the entire length of the horizontal slide. This movement will spread the blood out into a thin layer. Allow it to dry. Then immerse the slide in **Jenner's blood stain.** This is prepared as

follows: Mix equal parts of a 1.25 per cent. solution of Grübler's water-soluble eosin and 1 per cent. aqueous solution of *pure* methylen blue, after twenty-four hours filter, wash the precipitate with water, and dry. Make a ½ per cent. solution of this powder in pure methylen alcohol.

Allow the slides to remain in the staining fluid for from three to five minutes; then remove and wash thoroughly in water; allow the spread to dry, and mount in **balsam.** Place two drops of balsam, at some distance from each other, on the slide and put a cover-glass on each.

High Power.—Identify and make drawings of each form of the white cells.

The nuclei of the white cells will be stained blue, their cell bodies and the red cells reddish, the granules of the eosinophile cells bright red.

The eosinophile cells being few in number, careful search must be made for one.

EPITHELIAL TISSUES.

Epithelial tissue is composed of **cells,** which are close together and united by a slight amount of **intercellular substance.**

Cells.—Each cell has a nucleus, which is generally embedded in the cytoplasm. There are a few exceptions to this general rule, which will be noted when the special cells are considered.

The **cytoplasm** is soft and may be clear, finely or coarsely granular. It yields readily to pressure, consequently we have a great diversity in the shape of the cells.

Intercellular Substance.—The intercellular or cement substance is in the form of thin layers between the cells, fastening them firmly together. When the fresh tissue is treated with silver nitrate it unites with the intercellular substance, and if then the specimen be exposed to the light the silver becomes reduced and outlines the form of the cells with thin black lines.

Membrana Propria, or Basal Membrane.—All epithelium rests on a thin, structureless membrane, the membrana propria. This membrane is believed to be of connective-tissue origin; by some it is said to be formed by the epithelial cells.

Cuticula.—The cuticula is the thickened free surface of some epithelial cells, being sharply differentiated from the remainder of the cytoplasm. It is found chiefly on the surface of the cylindrical-shaped cells, and on those lining the small intestine it shows a distinct longitudinal striation. This striation is believed to be caused by fine prolongations of the cytoplasm into the cuticula.

Cuticular Membrane.—The fusing of the cuticula of adjoining cells forms the cuticular membrane.

As a general rule, blood vessels are not found in epithelial tissue. There is one exception, the **stria vascularis** of the cochlea. The nerve supply is rich.

CLASSIFICATION.

1. Simple epithelium. Consisting of one layer of cells.
   - *(a)* Squamous, or scale-like.
   - *(b)* Cylindrical or columnar.
2. Stratified epithelium. Consisting of several layers of cells.
   - *(a)* Squamous.
   - *(b)* Transitional.
   - *(c)* Cylindrical or columnar.
3. Modified
   - *(a)* Ciliated.
   - *(b)* Mucous or goblet.
   - *(c)* Pigmented.
4. Specialized.
   - *(a)* Gland epithelium.
   - *(c)* Neuro-epithelium.

Simple Squamous Epithelium.—The cells of this form of epithelium are laid down in a single layer. They are thin and scale-like, and irregular in shape: When viewed on the surface they show a mosaic arrangement; when on the edge they show a bulging of the cytoplasm at the situation of the nucleus, with a thinning out toward the edges, giving the cell a fusiform shape.

This type of epithelium is found in but few places in the body and will be studied with the organs in which it is situated.

Simple Cylindrical Epithelium.—The form of the cells in this class of epithelium is long and narrow, with a rounded, pointed, or forked base. The nucleus, oval in shape, is embedded in the cytoplasm of the cell near its base. The cuticula is well developed in the epithelium which covers the mucous membrane of the small intestine.

The height of this form of epithelium varies, sometimes being high, sometimes low, and in a few instances the height and width of the cell are about equal, the nucleus being embedded in the centre of the cytoplasm. This is known as **cuboidal epithelium.**

Stratified Squamous Epithelium.—The cells in this form of epithelium are laid down in several layers, their shape varying in each layer. Those of the deep layer are cuboidal or low cylindrical, with spherical-shaped nuclei; those of the middle layers irregular, with spherical or oval-shaped nuclei; those of the sur-

face alone being flat or scale-like, with elongated, oval-shaped nuclei. When seen in section, cut perpendicular to the surface, these squamous cells are fusiform in shape, with elongated nuclei.

This form of epithelium rests on a layer of connective tissue which is thrown up into a series of round or conical-shaped projections—**the papillæ.** These papillæ and the spaces between them are covered with the basal membrane.

In the epidermis of the skin, which is formed of this type of epithelium, the surface cells become hornified and lose their nuclei. In the middle layers the **spine** or **prickle** cells become developed.

Transitional Epithelium.—This is a stratified form of epithelium, the cells being laid down in from four to six layers. The cells are large and flat, their free surface being convex, their under surface pitted; this pitting being caused by the growing cells underneath pushing their heads into the cytoplasm of the overlying cells. The surface cells have large, spherical or oval-shaped nuclei. The cells of the deep layer are of an irregular cuboidal shape, with spherical-shaped nuclei; those of the middle layers pyriform or polyhedral, with roundish nuclei.

This form of epithelium is laid down on a layer of connective tissue *without papillæ.*

Stratified Cylindrical Epithelium.—The surface cells alone, in this form of epithelium, are of a cylindrical shape, their basal ends being pointed or forked. The nuclei, oval in shape, are situated near the base of the cells. The cells of the middle layers are irregular in shape, with roundish nuclei; those of the deep layer cuboidal, with spherical-shaped nuclei. The cuticula is in most cases well developed.

Ciliated Epithelium.—This may be either of the *stratified* or *simple* type, the cells in man being of the cylindrical shape. In the stratified form the surface cells are alone ciliated; the arrangement of the other cells being the same as stratified cylindrical epithelium. The simple form consists of a single layer of ciliated cylindrical epithelial cells.

The free surface of the ciliated cells has a well-marked **cuticula,** from which the **cilia**—hair-like appendages—apparently spring. The cilia are filamentous prolongations of the cytoplasm through and projecting beyond the cuticula.

The length of the cilia varies. In some places the cells have short and in others long cilia.

The number of cilia to a single cell also varies, being from 12 to 25.

The **movement** of a single cilium resembles somewhat that of a whiplash. It is automatic, not being under the control of the nervous system. The number of vibrations per second is estimated to be 10. The vibrations of the cilia do not take place in all of the cells at the same time, but pass through a series of cells in successive waves, one cell after another taking up the movement. The rapidity of the movement is increased by warmth, up to a certain point; it is decreased by cold.

Mucous Epithelium.—Mucous cells are found distributed among cylindrical epithelium in many parts of the body. They are numerous in the epithelium covering the surface of the mucous membrane of the trachea and intestine.

They are formed from the cylindrical cells, the most of the cytoplasm of which becomes converted into *mucin.* The process begins at the surface of the cell and progresses toward the base, until the bulk of the cell becomes transformed into mucin. The cell now becomes enlarged, generally oval in shape, and clear in appearance, a small amount of unconverted cytoplasm with the nucleus being crowded to its base. The free surface of the cell ruptures and its contents, *mucus,* is discharged. The cell then collapses, and from the remains of the cytoplasm and nucleus a new cell is formed.

Pigmented Epithelium.—In this form of epithelium the cytoplasm is more or less filled with granules of pigment. These granules vary in shape and color. In some cells they are spherical, in others rod-like; the color in some being light brown, in others dark brown or black.

Glandular Epithelium.—This is a form of epithelium lining the secreting portion of glands. The characteristics of the cells vary in different glands, and we will leave its consideration until we study the structure of glands.

Neuro-Epithelium.—This is a very highly differential epithelium found in the sense organs. We shall leave its consideration until they are studied.

MESOTHELIUM AND ENDOTHELIUM.

Mesothelium.—The original body cavity, in the embryo, is lined with flat cells partaking of the character of epithelial cells. In adult life cells derived from these line the cavities of the pleura, peritoneum, and pericardium. For these cells the term mesothelium has been suggested by Minot.

Mesothelial Cells are flat cells resembling very closely those of squamous epithelium. The cytoplasm is slightly granular. The nucleus, ovoid in shape, is situated in the central portion of the cell and projects above its free surface. The edges of the cell are wavy or serrated, and the cells are united by a very thin line of intercellular substance.

Endothelial Cells.—A similar type of cell to the mesothelial forms the walls of capillaries, lines the interior of blood vessels, lymph vessels, synovial spaces, bursa, tendon sheaths, and the anterior chamber of the eye. These are differentiated *mesochymal cells* (Minot), and now it is suggested that the term endothelium be applied to them alone.

These cells, as regards structure, are the same as the mesothelial cells. In form they are irregularly oblong, their edges being more deeply serrated than the mesothelial. Both of these types of cells are laid down in a single layer.

Practical Study.

Stratified Squamous Epithelium.—Pieces of the œsophagus of a recently killed animal are pinned out on a bit of sheet cork, mucous membrane uppermost, and fixed in formalin-Müller's fluid. After hardening in alcohol, the mucous membrane is dissected away from the outer coats. Pieces are then embedded in celloidin and sections made perpendicular to the surface of the mucous membrane.

Sections are stained double (see page 26) and mounted in **eosin-glycerin.**

Low Power.—Note the rows or layers of the epithelial cells, and the papillæ of the connective tissue projecting into them.

High Power.—Observe the shape of the cells in the various layers. Note that the deep layers of cells are cuboidal, with round nuclei; the cells change in shape as the surface is approached, the middle layers being large and irregular, with roundish nuclei; that the surface layers are flat or squamous and in section appear fusiform, with elongated nuclei. Observe that the cells are outlined by a narrow, clear line, the **intercellular substance.**

Also note the **basal membrane,** or **membrana propria,** not very distinct, being a narrow, clear-looking line upon which the cells are built up.

Make a drawing of a narrow section through the thickness of the specimen, showing the various layers of cells and their characteristics.

Simple Cylindrical Epithelium.—Small pieces of the small intestine are fixed and hardened as described under stratified squamous epithelium. Then embed in celloidin, and cut sections perpendicular to the surface of the mucous membrane.

Sections are **stained double** (see page 26) and mounted in **eosin-glycerin.**

Low Power.—Note that the surface of the section is not smooth, but is covered with numerous finger-like projections, the *villi,* and that these villi are covered with a layer of epithelial cells. Select a place where the cells are cut parallel to their long axis, and turn on the

High Power.—Observe the shape and dimensions of the cells; their thickened free border or **cuticula;** and that the cuticula of adjoining cells, fusing, forms the **cuticular membrane;** also note that the cuticula is longitudinally striated; and the shape and position of the nucleus.

Next observe the **mucous** or **goblet** cells, a form of *modified* epithelium. Note that these cells are scattered through the layer of cylindrical cells; that they appear as clear, oval-shaped spaces, and that by careful focussing a distorted nucleus can be seen at the base of the cell. Also note that some of the mucous cells

show a cloud-like mass of mucus projecting from their free surface.

Make a drawing of several cylindrical epithelial cells, showing their relations to each other and the details of structure.

Also make a drawing of a single mucous cell, showing its structure and its relations to the adjoining cylindrical-shaped cells.

Stratified, Cylindrical, Ciliated Epithelium.—Remove the trachea from a recently killed dog and cut it up, lengthwise, along its posterior surface. Pin it out securely on a piece of sheet cork, inner surface uppermost.

Macerate the epithelium by floating the specimen on Müller's fluid, diluted one-half with water, for 12 hours; then wash by floating the specimen on water. Then with a knife gently scrape the surface of the specimen to remove the cells. Place the scrapings in 80 per cent. alcohol contained in a tall, cylindrical-shaped vessel, and allow them to settle. Then pour off the alcohol and add a sufficient quantity of alum-carmin (see page 22) for staining the nuclei. Allow to stand for 12 hours, and then wash with water by decantation. Finally preserve in **eosin-glycerin.** Take up a drop of the glycerin containing the cells, place it on the centre of a slide, and put on a cover-glass.

Low Power.—Observe that the specimen consists of isolated cells and cells in clumps. Place one of these clumps in the centre of the field of the microscope and turn on the

High Power.—A. Select a mass of the cells which shows several layers (stratification). Note the cylindrical-shaped surface cells and that their free surface is *ciliated.* Note the size, shape, and relations of the underlying cells, also their relation to the surface cells. Make a drawing of the mass of cells, showing all details.

B. Select an isolated surface cell and make a drawing of it, showing all details.

C. Select a *mucous cell* and make a detailed drawing of it.

Living Ciliated Cells.—For studying living ciliated cells, bits from the gill of an oyster are used.

Place a large drop of *sodium chlorid* solution on the centre of a slide; then, having opened the shells of an oyster, with a pair of small scissors snip off a bit of the gill. Place it in the salt solu-

tion on the slide, and with the teasing needles gently tease it apart. Cover with a cover-glass and place the slide on the stage of the microscope.

Low Power.—Select a thin place in the specimen, where a mass of the cilia are in motion, and observe their action.

High Power.—Select a single, living cell and study the action of the cilia. Note that these cells are of the spherical order and not cylindrical.

Pigmented Epithelium.—An ox's eye is hardened in Müller's fluid for 4 to 5 days. It is then opened by an equatorial section and the vitreous and retina removed from the posterior half. The most of the retinal cells will remain attached to the inner surface of the choroid coat, and are removed by gently scraping them away with a knife. Preserve in **pure glycerin.**

Place a drop of the glycerin on the centre of a slide and examine with the

Low Power.—Select a group of the cells, note the hexagonal shape and that they are more or less filled with pigment granules.

High Power.—Observe that some of the cells are crowded with pigment granules (note their color and shape); others are quite free and others entirely free from pigment granules. Note that the nucleus is unstained and appears as a circular spot in the centre of the clear cells; in the deeply pigmented cells it appears as a semi-clear spot, or it may not show, being covered with the pigment granules.

Make a drawing of three or more of the pigmented cells, showing their shape and the shape of the pigment granules.

Omentum of the Dog Treated with Silver Nitrate.—A portion of the omentum is removed from a recently killed dog and handled so as all stretching and pulling is avoided. It is placed in a large dish filled with water to remove any blood or albuminous substance on its surface, which would cause a granular precipitate of silver. The water is poured off the specimen and replaced with a 1 to 500 solution of silver nitrate in distilled water. The dish should be shaken at intervals, so that fresh silver solution will be brought in contact with the specimen. After allowing the silver solution to act for from twenty minutes to half an hour, it is poured off and the specimen washed with water.

The specimen is then placed in 80 per cent. alcohol and exposed to the sunlight until it becomes of a light-brown color. It is then transferred to fresh 80 per cent. alcohol for preservation.

The specimen is floated out on water, and small pieces of the thin portions are cut out with scissors, stained with **hæmatoxylin,** and mounted in **pure glycerin,** as follows: A bit of the omentum is put in water, from alcohol, when it will flatten out. Introduce a slide, holding it perpendicular, into the water; then with the point of a teasing needle bring the specimen to the centre of the slide and fix it there by firm pressure with the needle. Withdraw the slide from the water, keeping it in a perpendicular position; as it is drawn out of the water the specimen will flatten down on it. Absorb the water with bits of filter paper, taking care not to touch the specimen; place a drop of **pure glycerin** on it and put on a cover-glass.

Low Power.—Note the structure of the omentum; that it consists of an irregular meshed net, the **trabeculæ** of which are various-sized cords of connective tissue, all of which are covered with a single layer of flat cells—**mesothelial cells.** The outlines of the cells are marked out by thin black lines, the reduced silver in the intercellular substance.

Make an outline drawing showing the general structure of the omentum.

High Power.—A. Select one of the **broad trabeculæ.** Observe the mosaic arrangement of the cells, the outlines of which are marked out by the thin, finely granular, black lines. Note the nuclei of the mesothelial cells stained blue and the more faintly appearing nuclei, the nuclei of the underlying connective tissue cells.

Make a drawing showing the details of the structure.

B. Select one of the **thin trabeculæ.** Observe the outline of the cells and that in many places the cells wrap themselves around the trabeculæ. Also note that in some places the nuclei project into the clear spaces of the net, demonstrating that the nuclei project above the surface of the cells.

Make a drawing showing all the details of structure.

Endothelial Cells of Blood Vessels.—The endothelial cells lining the blood vessels are best demonstrated in the bladder of

the frog. The bladder is exposed in a freshly killed frog, and a glass canula passed into it; the organ is slightly distended with air and then ligated. It is then cut out and immersed for twenty minutes in a ½ per cent. solution of silver nitrate in distilled water. It is then treated in the same manner as the omentum.

CONNECTIVE TISSUE.

Connective tissue is one of the important tissues of the body on account of its wide distribution. It is subdivided into several groups. The physical characteristics of these subgroups vary, some being loose tissue, others, like bone and cartilage, dense.

An important characteristic of connective tissue is that its various groups are capable of being transformed into entirely different types; bone, for instance, being developed from fibrillar connective tissue and cartilage.

Its elements, like other tissues, consist of **cells** and **intercellular substance.**

Classification.

The members of the connective-tissue group, while presenting great differences in form, are, as regards structure, identical. It may therefore be divided into the following groups:

1. Fibrillar connective tissue.
2. Embryonal tissue.
3. Mucous tissue.
4. Fat tissue.
5. Cartilage.
6. Bone and teeth.
7. Reticular connective tissue.

Fibrillar connective tissue is composed of **intercellular** or **ground substance** and **cells.**

The **intercellular substance** consists of two kinds of fibres, **fibrillated fibres** and **elastic fibres.**

The **fibrillated fibres** are composed of fine fibrils packed together and united by a small amount of **interfibrillar cement substance.** The fibrils are laid down parallel to each other. The fibres vary in size and have a distinct longitudinal striation. Their

shape is, generally, that of a flattened cord. They never branch or anastomose with each other.

On boiling in water they become converted into gelatin. When treated with hydric acetate or weak solutions of alkalies, they swell up and become transparent.

The **elastic fibres** are homogeneous and highly refractive. They vary in size from 1 μ[1] to 10 μ in diameter. The smallest fibres are round in shape, while the larger, on transverse section, appear flattened or hexagonal in shape. They branch and form anastomoses with adjoining fibres. Upon being broken across they tend to bend or curl at their broken ends. When extended they appear as straight lines, but when relaxed they assume a curved or spiral arrangement. Boiling in water does not convert them into gelatin, but into a substance known as *elastin*. They are not affected by hydric acetate, but are destroyed by the action of a 50 per cent. solution of hydric chlorid. Strong solutions of potassium hydrate cause them to disintegrate in the course of a few days. Pancreatin and trypsin dissolve the fibres, the latter more slowly.

According to Mohl, elastic fibres are composed of two distinct substances—an external, delicate, homogeneous membrane which encloses a somewhat granular material which he has named *elastin*. Solution of magenta (an anilin dye) stains the elastin intensely, but does not color the external membrane.

The **cells** are of four varieties—**fixed cells, wandering cells, plasma cells,** and **mast** or **granular cells.**

The **fixed connective-tissue cells** are of a flattened, irregular shape with many branches; when seen on edge or in sections cut perpendicular to their surface, they have a fusiform shape. The cytoplasm is generally clear or very finely granular. The nucleus, situated in the thicker portion of the cell, is oval in shape, having a well-marked intranuclear network and one or more nucleoli. These cells are situated in **cell spaces,** which they almost completely fill. These cell spaces are connected by minute channels, the **canaliculi,** and the processes of adjoining cells often extend

[1] The unit for microscopic measurement as now adopted is the micro-millimetre, or the one-thousandth part of a millimetre. It is called a *micron*, and the Greek letter μ is used as its symbol.

out into these canaliculi and anastomose with processes of other cells.

(1) The **wandering connective-tissue cells** are the *lymph* or *white blood cells* which have left the vessels and, by means of amœboid movement, crawl through the cell spaces and canaliculi. They are spherical-shaped, nucleated cells.

(2) The **plasma cells** are spherical-shaped cells, the cytoplasm being coarsely granular and containing a number of vacuoles. They are found in the neighborhood of the blood vessels.

(3) The **mast cells** are spherical or irregular in shape, the cytoplasm being filled with coarse granules, which stain intensely with the anilin dyes. They are found near the blood vessels and are supposed to have some connection with the formation of fat.

It has been suggested that the plasma and mast cells are not connective-tissue elements, but differentiated white blood cells.

Like epithelial cells, connective-tissue cells may be **pigmented.** Such a cell is found, in man, in the iris, choroid, and skin. They are irregular-shaped cells, the cytoplasm being more or less filled with pigment granules of a brown or black color.

Morphologically, fibrillar connective tissue may be divided into two groups, **areolar tissue** and **tendons, aponeuroses** and **ligaments.**

Areolar tissue is composed of various-sized fibrillated fibres which, crossing each other in various directions, form a network with large, irregular meshes, making a loose tissue. The meshes of the tissue are filled by a semi-fluid, homogeneous substance, the **ground substance** or **matrix.** In a more dense form of areolar tissue the fibres replace the ground substance. The cell spaces with their connecting canaliculi are in this ground substance.

Tendons, aponeuroses, and **ligaments** are dense forms of fibrillar connective tissue and are made up almost entirely of the white or fibrillated fibres.

Tendons are made up of fibres which run parallel to each other. Aponeuroses and ligaments have practically the same structure as a tendon, only they are much wider, the former in many instances forming sheets.

PRACTICAL STUDY.

Fibres of Subcutaneous Tissue (Areolar Tissue).—In order to study these fibres it is necessary to have fresh subcutaneous tissue. An animal is killed and the skin removed from the abdominal wall. A bit of the loose areolar tissue, free from fat, is picked up with the points of a pair of forceps and cut off with a pair of scissors. Transfer it to the surface of a clean, dry slide, and with the points of the teasing needles stretch it out to nearly the length of the slide; then with the needles stretch it crosswise of the slide until it is reduced to an exceedingly thin layer. *All of these manipulations must be done as quickly as possible. Breathe on the specimen occasionally, during the manipulations, to prevent it from drying.*

Finally put a drop of **sodium chlorid solution** on the specimen and put on a cover-glass.

Low Power.—Find a thin place in the specimen and turn on the

High Power.—A. Observe the **white** or **fibrillated fibres**; note their difference in size; that they may be round or flattened; that they are longitudinally striated.

Make a drawing of one of the fibres.

B. Observe the thin, thread-like **elastic fibres**; note their homogeneous appearance; that they give off branches which anastomose with other fibres; that their broken ends curl.

Make a drawing showing the above details.

C. Place a drop of **hydric acetate (2 per cent.) solution** in contact with one edge of the cover-glass, and a bit of filter paper in contact with the opposite edge. The filter paper will suck out the salt solution from under the cover, and the hydric acetate will take its place. After a few minutes examine with the high power and note the action of the hydric acetate on the fibres. Which fibres does it affect? fibrillae get transparent

This specimen is not to be preserved.

Elastic Fibres from the Ligamentum Nuchæ.—This ligament is composed almost entirely of elastic fibres. A piece of the

ligament is fixed in a saturated aqueous solution of picric acid and then hardened in alcohol.

A small piece is teased apart in **glycerin** and mounted in the same medium.

Low Power.—Select a single fibre and turn on the

High Power.—Observe that the fibres are much coarser than those seen in the subcutaneous tissue, and that they appear somewhat flattened. Note the broken branches and curled ends.

Make a drawing showing the appearance of a single fibre.

Connective-Tissue Cells in Subcutaneous Tissue.—This specimen is prepared in the same manner as the previous one, except after spreading out the tissue it is allowed to dry on the slide. When it has become perfectly dry flood the entire specimen with the following staining fluid: saturated alcoholic solution of gentian violet, 40 c.c.; water, 60 c.c.

Allow the stain to act for 4 to 5 minutes; then wash off the stain by moving the slide about in the tumbler of water.

Stand the slide on end to drain and dry.

Place a drop of balsam on the specimen and put on a cover-glass.

High Power.—Select a thin place in the specimen. Note the **nuclei** of the cells, stained a deep violet color, and the thin, irregular-shaped **cytoplasm,** finely granular, surrounding the nucleus as a faintly stained mass. Look over the specimen and see if a wandering cell, a plasma cell, and a mast cell can be found. Also note the **elastic fibres,** stained deep violet, and the **fibrillated fibres,** very slightly colored. Note the peculiar appearance of some of the nuclei; that they are crossed by one or more clear lines. What causes this appearance?

Make a drawing of the various kinds of cells.

Pigmented Connective-Tissue Cells.—An eye of an ox is hardened in Müller's fluid for a week. The eye is then cut in two and washed in water. Then remove the **choroid coat** and pick off bits of its outer layers. These are stained with **hæmatoxylin** and mounted, as described under the omentum of the dog, in **pure glycerin.**

Low Power.—Note the irregular shape of the cells and that the cytoplasm is filled with pigment.

High Power.—Select a thin place in the specimen. Note the shape of the cell body; the shape and color of the pigment granules; and the free nuclei. What are they?

Make a drawing of a single cell showing all details of structure.

EMBRYONAL AND MUCOUS TISSUE.

Embryonal Tissue.—In the early stages of the growth of the embryo the cells of the connective tissue are all spherical in shape, the intercellular substance being but slight in amount and fluid. As development goes on some of the spherical cells begin to change in shape, becoming oval, then elongated and fusiform. While the changes are taking place in the cellular elements, the intercellular substance begins at first to increase in quantity, remaining at first fluid, later becoming gelatinous and homogeneous in appearance. Soon delicate fibrillæ begin to appear and increase in number.

When the developing connective tissue reaches the stage where the **majority of the cells are of a fusiform shape** and the intercellular substance contains fibrils, the name **embryonal tissue** has been applied to it.

Mucous Tissue.—This is an older form than the former. It is not found normally in the adult human body. It consists of branched, anastomosing cells, which form a network, embedded in a matrix of gelatinous substance containing a few white or fibrillated fibres. The matrix contains *mucin*. It is found in large quantities in the umbilical cord of the fœtus and is here known as the **jelly of Wharton.**

Development of Fibres.—There are two theories as to the manner of the development of the connective-tissue fibres: 1. Their origin from cells. 2. Their origin from the matrix.

Origin from Cells.—Certain of the cells undergo a differentiation; they become elongated and a delicate longitudinal fibrillation appears in their cytoplasm. These cells are the **fibroblasts of Ziegler,** and from them the fibrillated fibres are developed.

Origin from the Matrix.—In this form of development granules appear in the matrix and become arranged in rows. The

granules fuse, forming fibres. This is probably the way in which elastic fibres are formed.

The intercellular formation of fibres is the more usual.

PRACTICAL STUDY.

Subcutaneous Tissue of Fœtal Pig.—Fœtal pigs five inches in length[1] are taken and bits of the gelatinous tissue found in axilla and groin are cut out with scissors and fixed in Zenker's fluid (see page 15). After hardening in alcohol the nuclei are stained with alum-carmin by immersing the bits of tissue in the stain for 12 hours. Then wash well in water and preserve in **eosin-glycerin.**

A small bit of tissue is placed on a slide in a small drop of **eosin-glycerin.** It is then **teased** up into fine bits with the teasing needles and a cover-glass put on.

Low Power.—Select a thin bit of the tissue and place it in the centre of the field of the microscope and turn on the

High Power.—Observe the shape of the cells, especially the **fusiform** ones; note the character of the intercellular substance.

Make a drawing of the various forms of the cells and of the intercellular substance.

Mucous Tissue in the Umbilical Cord.—The umbilical cord of a human fœtus of about three to four months, or of a fœtal pig eight to nine inches long, is fixed in formalin-Müller's fluid, hardened in alcohol, and embedded in celloidin.

Transverse sections are made through the cord, stained **double,** and mounted in **eosin-glycerin.**

Naked Eye.—Observe the shape of the section, and that in its central portion three roundish masses are to be seen, the umbilical blood vessels.

Low Power.—Observe that the edge of the section is surrounded by a layer of epithelial cells, and that the blood vessels are surrounded by concentric layers of tissue. Pass near the

[1] We have no means of telling the age of a fœtal pig except by its length. This is determined by measuring it, along its back, from the tip of the nose to the base of the tail.

edge of the section, away from the blood vessels where the **pure mucous tissue** is to be found, and turn on the

High Power.—Observe the irregular-shaped cells; their branches, which in many instances may be seen connecting with branches of other cells, forming a network. Note that some of the cells appear without branches. What is the reason for this appearance? Observe the character of the intercellular substance.

Make a drawing showing the *arrangement* of the cells; the *details* of their structure; and the *details* of the structure of the *intercellular substance.*

FAT TISSUE.

In order to understand the appearance of adult fat tissue it should be first studied in its **developing stage.**

Fat tissue first makes its appearance in the human embryo about the thirteenth week of intrauterine life, the fat cells being developed from the connective-tissue cells.

Clusters of young connective-tissue cells, the fat islands of Minot, or **primitive fat lobules,** gather in the vicinity of the blood vessels in the meshes of a capillary network, the cells being of a spherical or oval shape.

Small granules of **fat** appear in the cytoplasm, and the nucleus of the cell passes to its side. These fat granules fuse, forming small drops of fat; these fusing form larger drops of fat; these in turn uniting form one large mass of fat which occupies nearly the entire cell body. A distinct cell membrane is formed, and a small amount of cytoplasm containing a much-flattened nucleus is crowded to the periphery of the cell, forming a crescent-shaped mass just underneath the cell membrane.

Adult fat consists of small vesicles filled with an oily material, the fat cells.

These fat cells may occur in small groups, as single cells, or more commonly they are packed closely together in masses, the fat lobules, which are surrounded by a layer of fibrillated connective tissue. The connective tissue penetrates the lobule in the form of thin strands, in which numerous small blood vessels are found.

Single fat cells or small groups are generally spherical or oval in shape, but when they are packed together in the lobules they may assume various shapes, owing to the pressure of adjacent cells.

Osmic acid stains fat black.

Practical Study.

Developing Fat from Fœtal Pig.—A fœtal pig five inches in length is taken and bits of the gelatinous tissue removed from the axilla and groin. These bits of tissue are immersed in a 1 per cent. aqueous solution of osmic acid for 24 hours, then washed thoroughly in water, and preserved in **pure glycerin.**

A bit of the tissue is placed in a drop of **pure glycerin** on a slide and teased apart slightly with the teasing needles. A cover-glass is then put on and the specimen examined with the

Low Power.—Look over the specimen and see if any of the **primitive fat lobules** show. The fat in the cells will be stained black by the osmic acid.

If a primitive lobule is found, make a drawing of it showing its relation to the blood vessel.

High Power.—Select isolated cells and make drawings showing at least three stages of development.

Adult Fat Tissue.—The subcutaneous tissue of man with its layer of fat is hardened in formalin-Müller's fluid, washed well in water, and embedded in celloidin.

The embedding in celloidin must be done with care. The alcohol and ether dissolves out the fat from the cells, and this, mixing with the celloidin, forms a pasty mass. In order to obtain a thorough impregnation, the pieces of tissue should be small, half a cubic inch; the alcohol and ether and the thin celloidin should be changed several times before the specimens are placed in the thick celloidin.

Sections are stained in a 1 per cent. aqueous solution of **acid fuchsin** for fifteen to twenty minutes; then washed in several changes of 97 per cent. alcohol, cleared in oil of origanum, and mounted in balsam.

The acid fuchsin stains the cell membrane of the fat cells and all connective tissue a bright red. Sometimes the nuclei of cells are also stained red.

Low Power.—Observe the grouping of the fat cells into lobules, each of which is surrounded with a band of connective tissue which sends thinner septa into the interior.

Make a drawing of a lobule showing its structure.

CARTILAGE.

From the character of the intercellular substance, cartilage is divided into three varieties: **hyalin, elastic,** and **fibrocartilage.**

General Structure.—Cartilage is firm, highly elastic, yields to pressure, but readily returns to its normal shape when the pressure is removed.

The **cells** may be round, oval, or bluntly angular in shape. The cytoplasm is finely granular; sometimes it shows a filamentous structure. The nucleus is spherical in shape, its intranuclear network being rather coarse. A cell may have two nuclei. The cells lie in **cell spaces** lined with a **capsule,** and which they completely fill. The cell spaces are believed to be connected by a series of fine canals, the *canaliculi.*

The **intercellular** substance varies in its physical characteristics in each variety.

Hyalin Cartilage.—The intercellular substance, by the ordinary methods of preparation, appears homogeneous, but by special methods it has been demonstrated that it is composed of bundles of fibres.

The cells are usually grouped in clusters of two and four, but many single cells also occur.

Hyalin cartilage, except where it forms the articular surfaces of joints, is surrounded by a connective-tissue membrane, the **perichondrium.** It occurs in the trachea, bronchi, nose, costal cartilages, and covers the articular surfaces of joints. In the embryo it forms the matrix of many of the bones.

Elastic Cartilage has a yellow color. Its intercellular substance consists of fine elastic fibres, which form a network and surround the cell spaces. It has a perichondrium which blends with its intercellular substance. It is found in the epiglottis, some

of the cartilages of the larynx, in the Eustachian tube, and external ear.

Fibrocartilage has an intercellular substance composed of fibrillated connective-tissue fibres. The cell spaces are few in number, generally elongated in shape, and may contain two or more cells. It occurs in the intervertebral discs and around the edges of cup-like articulations.

Practical Study.

Hyalin Cartilage.—A frog is killed, the femur disarticulated at the hip joint, and its head immersed immediately in a saturated aqueous solution of picric acid. Sections are cut under picric acid solution and mounted in the same. A drop of the above picric acid solution is placed on a slide, a section of the cartilage is placed in it, and a cover-glass put on. The cover-glass is to be cemented to the slide immediately. A saturated aqueous solution of picric acid preserves the normal appearance of the cells better than any other reagent. Most all the other fixatives cause the cells to shrink away from the capsule of the cell space and become contorted.

Low Power.—Observe the grouping of the cartilage cells, and note that in some cases the cells may have dropped out of the cell spaces, leaving clear cavities.

Make a drawing showing the grouping of the cells.

High Power.—Observe the structure of the cartilage cells. Note that they completely fill the cell spaces. Also note the capsule of the cell space, and, finally, the appearance of the intercellular substance.

Make a drawing showing the details of structure of a cell and the intercellular substance.

Elastic Cartilage.—The human epiglottis is removed and fixed in *picrosulphuric acid* for twelve hours, then washed thoroughly in water and hardened in alcohol. Embed in celloidin and make longitudinal sections through the specimen.

The sections are stained **double** and mounted in **eosin-glycerin.**

Low Power.—Observe the plate of elastic cartilage embedded

in the central portion of the specimen, and that it is surrounded by a **perichondrium.**

High Power.—Note the structure of the intercellular substance. Observe the cell spaces and that they contain shrunken cartilage cells. Pass to the edge of the cartilage and note the *perichondrium* and the zone of flattened cells just under it.

Make a drawing showing all details.

Fibrocartilage.—Pieces of the intervertebral disc are fixed in formalin-Müller's fluid, hardened in alcohol, and embedded in celloidin.

Sections are stained **double** and mounted in **eosin-glycerin.**

High Power.—Observe the character of the intercellular substance, and the cells.

Make a drawing showing the details of structure.

If time permits relation of intervertebral disc to the bone

BONE.

Bone is a form of connective tissue in which the intercellular substance is impregnated with lime salts, making it hard and dense.

Dried bone is composed of one-third organic matter and about two-thirds mineral matter. The mineral matter consists of neutral calcic phosphate, 57 per cent.; calcic carbonate, 7 per cent.; magnesic phosphate, 1. to 2 per cent.; calcic fluorid, 1 per cent.; with traces of chlorin.

The **bone cells** are situated in cell spaces, or **lacunæ.** These lacunæ are connected by a series of fine canals, or **canaliculi,** which pass off from them in all directions, and open into the marrow cavities or into channels containing blood vessels. The bone cells are thin, flat, nucleated cells.

Bone occurs in the form of either **spongy** or **cancellous,** or **compact.**

Spongy or **cancellous bone** occurs in the heads (epiphyses) of the long bones and between the tables of the flat bones. It is formed of thin plates, grouped so as to form small, irregular-shaped spaces which are filled with marrow.

Compact bone forms the shafts (diaphyses) of the long bones, and its intercellular substance is laid down in layers, or **lamellæ,** in which are the bone cells in their lacunæ.

Compact bone contains a series of channels or canals, the **Haversian canals.** These Haversian canals anastomose with each other, forming an irregular network. They vary in size and they open into both the marrow cavity and upon the surface of the bone. They contain blood vessels, and the larger ones marrow. These canals for the most part run parallel with the surface of the bone.

The **lamellæ** of compact bone are laid down in three general systems: the **Haversian,** the **interstitial** or **ground,** and the **circumferential lamellæ.**

The **Haversian systems** are grouped around the Haversian canals. When seen in transverse section they appear as concentric layers with the Haversian canal as the centre. These systems vary in size and lie at varying distances from each other. The lamellæ appear as if separated by thin lines and the lacunæ are embedded in them.

The **interstitial** or **ground lamellæ** are generally short and join the Haversian systems to each other and to the circumferential lamellæ.

The **circumferential lamellæ** are of two kinds: the **outer,** which lie under the periosteum, and the **inner,** those surrounding the medullary canal.

Sharpey's fibres are bundles of fibrillated fibres, which may or may not be calcified, and which perforate the lamellæ either obliquely or at right angles.

Volkmann's canals are channels passing through the circumferential lamellæ and are not surrounded by concentric lamellæ. They convey the blood vessels from the periosteum to the Haversian canals.

The **periosteum** is a connective-tissue membrane which covers the bones externally and is firmly adherent to them. It is composed of two layers: the outer, which consists of coarse connective-tissue fibres and contains the large blood vessels; the inner, which consists of numerous fine elastic fibres and the smaller blood vessels. The tendons and ligaments are firmly attached to the periosteum.

The **marrow** occupies the cavity of the **medullary canal** and larger Haversian canals of the long bones and the spaces between the trabeculæ of cancellous bone. It is of two varieties, the **red marrow** and the **yellow marrow.** The marrow in young bones, in the vertebræ, ribs, sternum, and bones of the skull is of the *red* variety. The adult long bones contain the *yellow* variety. The difference in color is due to the quantity of fat, yellow marrow consisting almost entirely of fat.

Marrow is surrounded by a delicate connective-tissue mem-

brane, the **endosteum.** Its tissue consists of a reticulum of connective tissue in the meshes of which are embedded several varieties of cells.

The cellular elements of red marrow are as follows:

The Marrow Cells, or Myelocytes.—Cells slightly larger than a leucocyte, with a large, spherical or oval-shaped nucleus, containing a small amount of chromatin. The cytoplasm is slightly granular, the granules staining with the neutrophile dyes.

The Nucleated Red Blood Cells.—These are of two classes, the **erythroblasts** and the **normoblasts.**

The **erythroblasts** are the younger form of the two varieties. They have quite a large nucleus with a distinct intranuclear network. The cytoplasm resembles that of a red blood cell and contains a slight amount of hæmoglobin. They often show mitotic changes in the nucleus.

The **normoblasts** are developed from the erythroblasts. Their nuclei are spherical in shape; there is no demonstrable intranuclear network, though the nucleus stains deeply as a whole. The cytoplasm resembles that of a red blood cell and contains a considerable amount of hæmoglobin. The normoblasts are converted into red blood cells by the extrusion of the nucleus.

The Eosinophile Cells.—These are probably the same as the eosinophile leucocytes of the blood. Some of these cells have round, others horseshoe nuclei.

The Myeloplaxes, or Giant Cells.—These are large, polynucleated cells whose function is unknown. They possess the power of amœboid movement. They probably originate from a single leucocyte by an increase in its size, and not by the fusing of several leucocytes as has been supposed.

Practical Study.

Decalcified Bone.—A fresh metacarpal bone of an adult is fixed in formalin-Müller's fluid, washed in water, and hardened in alcohol. The lime salts in the intercellular tissue are removed by treating the bone with an acid, **decalcifying** it. For this purpose the following **decalcifying fluid** is used: A cold saturated solution of sodium chlorid is diluted with 2 volumes of water

and then 2 per cent. of hydric chlorid added. This fluid is changed daily until the specimen becomes decalcified; this is determined by passing a needle through it. It is then washed in a half-saturated aqueous solution of sodium chlorid, which is changed daily until all traces of the acid are removed. The addition of a few drops of ammonium hydrate will hasten the process. The bone is then placed in 97 per cent. alcohol for 24 hours and then embedded in celloidin. Both longitudinal and transverse sections of the bone are made, stained **double**, and mounted in **eosin-glycerin.**

Transverse Section. Low Power.—Observe the periosteum adherent to the surface of the bone. Note that the latter is not smooth, but irregular, and that the **periosteum** dips down into these irregularities.

Observe the various systems of **lamellæ**; the **Haversian systems** grouped around the circular or oblong openings, the **Haversian canals**; the **interstitial** or ground lamellæ connecting the other systems; the **circumferential lamellæ** under the periosteum. Note the **lacunæ** and the nuclei of the contained **bone cells,** stained with the hæmatoxylin.

Make a drawing showing the three systems of lamellæ and the periosteum.

Longitudinal Section.—Proceed as under the transverse section. Note that the Haversian canals are cut *longitudinally.*

Make a drawing showing the Haversian canals and the lamellæ.

Sections of Hard Bone.—One of the long bones is freed from its soft parts and macerated for several months in water, which must be changed frequently. It is then thoroughly washed and allowed to become perfectly dry. Then, with a fine saw, sections 1 mm. thick are cut. One of the surfaces of the section is ground smooth with emery and water, spread on glass plate, then polished on a hone. This section of bone is then fastened to a block of glass or wood, polished side down, with red sealing wax. Then with a coarse file, which is to be followed by a fine one, file the bone down until the sealing wax can be seen through it. The bone is then ground on a glass plate with emery and water until the thin section appears of a decided pink color from the shining through

of the red sealing wax. The bone section is removed from the block by soaking in alcohol. It is next polished by grinding on a fine hone slightly moistened with water. The section is then placed in strong alcohol, which is changed frequently until all traces of the sealing wax have been removed. It is then cut into small pieces (2 mm. square) and placed in ether for at least 24 hours.

One of these bits of bone is to be mounted as follows: A small bit of hard balsam (see page 24) is placed on the centre of a slide and the slide heated until the balsam becomes melted. Then one of the bits of the thin bone, which has been removed from the ether and allowed to become perfectly dry in the air, is picked up with the point of a teasing needle and plunged into the melted balsam. A cover-glass is put on as quickly as possible, pressed down, and the slide placed on a cold metal surface to chill the balsam. By this manipulation air becomes imprisoned in the **lacunæ** and **canaliculi** of the bone, and when examined with the microscope appears as black spaces and fine lines.

High Power.—Note the appearance of the **lacunæ** and **canaliculi.** It is possible that they may not show over the entire specimen, as some of the fluid balsam may have run into the lacunæ and canaliculi, rendering them invisible.

Make a drawing of two or more lacunæ and their connecting canaliculi.

Marrow.—The femur of a young child or animal (rabbit) is split open lengthwise and the cylinder of marrow in the medullary canal carefully removed. This is fixed in Zenker's fluid and hardened in alcohol. Embed in celloidin and make *transverse* sections. These are stained **double** and mounted in **balsam.**

A second method, whereby the cells become isolated, may also be used. Place a drop of fresh marrow squeezed from the rib on a clean cover-glass and cover it with a second. Make slight pressure on the covers, so as to spread out the marrow into a thin layer; then slide the covers apart and drop them at once in a saturated aqueous solution of mercuric chlorid. At the end of five minutes remove the covers and wash in water. Then stain the film of marrow by immersing the covers in *hæmatoxylin* for five minutes; wash in water, and stain for ten minutes in *eosin-alcohol;* wash quickly in water and allow the covers to become thoroughly

dry. Then mount by inverting the cover-glass on a drop of balsam placed on a slide.

Section of Marrow. Low Power.—Observe the arrangement and number of the cells. Note the large number of fat cells.

High Power.—Identify the various cells—the giant cells or **myeloplaxes,** the **marrow cells** or **myelocytes,** the **nucleated red blood cells,** the **leucocytes,** and the **fat cells.** See if one of the **eosinophile cells** can be found.

Make a drawing of the various kinds of cells.

DEVELOPMENT OF BONE.

At quite an early period of embryonic life the matrices of the bones of the adult body are laid down either in **connective-tissue membranes** or **embryonic cartilage.** From these matrices bone is developed in either one of the following ways: **intramembranous, intracartilaginous** or **endochondral,** and **subperiosteal** or **perichondral.**

Intramembranous Development of Bone.—This is the method by which the bones of the vault of the cranium and the greater number of the bones of the face are formed. The matrix of the bone is first laid down in a connective-tissue membrane. The process of the bone formation starts from one or more points, the **ossification centres,** and gradually spreads until the matrix becomes ossified. This process of ossification is carried on through the agency of special cells, the **osteoblasts.**

The connective-tissue fibres in the ossification centre become **calcified,** *i.e.,* lime salts are deposited in them. In the spaces formed by the connective-tissue fibres capillary blood vessels and **osteogenetic** tissue appear. Certain cells of the tissue become differentiated, forming the **osteoblasts.** The osteoblasts become arranged, in a single row, along the calcified connective-tissue fibres, and through their activity a layer or **lamella** of bone is formed around the fibre. These osteoblasts first form a thin layer of bone between themselves and the fibre; this increases in thickness; the osteoblasts become enclosed in a cell space, **the lacuna,** and become **bone cells.** The layer of new-formed bone with the enclosed bone cells becomes a **lamella** of bone. A new row of osteoblasts appear and a new layer of bone is formed. This process is repeated; the bone gradually increases in thickness, until the matrix is converted into spongy bone, its spaces being filled with embryonic marrow. These spaces usually contain a small blood vessel.

This process of bone formation continues toward the periphery until the connective-tissue membrane matrix becomes converted into cancellous bone. The bone continues to grow in thickness by deposition of new bone under the **pericranium,** and as this is proceeding the already formed bone in the inner surface is absorbed. This process of absorption takes place through the agency of large, polynucleated cells, the **osteoclasts.** These osteoclasts lie in depressions—**Howship's lacunæ**—on the edges of the trabeculæ, undergoing absorption.

Intracartilaginous Development of Bone.—This is the mode of development of all of the bones of the trunk, extremities, and a portion of the bones of the base of the cranium.

The **matrix** of these bones is first laid down in *embryonic* cartilage, which is surrounded by a membrane, the perichondrium. The embryonic cartilage becomes converted into hyalin cartilage, and the process of ossification begins at one or more points in the matrix, the **ossification centres.** In the long bones one of the centres of ossification is situated in the middle of the diaphysis.

At the commencement of the ossification process the cartilage matrix becomes *calcified* at the ossification centre. The cartilage cells enlarge, become grouped in a spherical mass, the peripheral cells of which become somewhat flattened.

At this stage a thin layer of bone is formed under the perichondrium and the blood vessels of the *osteogenetic* layer grow into the cartilage matrix. The septa of the matrix break down before the growing blood vessels, the cartilage cells disappear, and irregular communicating spaces, **the primitive marrow spaces,** are formed. These are separated by irregular partitions of cartilage matrix and are filled with **osteoblasts,** embryonic marrow tissue, and blood vessels.

The channelling out of the cartilage matrix by the growing blood vessels goes on until the **ossification centre** is reached, and it then continues toward the extremities of the diaphysis in the long bones.

The cartilage cells of the matrix at this stage become arranged in parallel rows. The advancing blood vessels cause an absorption of the matrix, the cartilage cells disappear, and a series of irregular channels, the **primitive Haversian canals,** are formed

The new bone is deposited on the partitions of cartilage matrix by the **osteoblasts**, in the same manner as it is on the connective-tissue fibres in the intramembranous development.

At a certain stage of the development the middle of the diaphysis consists of cancellous bone. This is absorbed by the action of the **osteoblasts** and the **medullary canal** is formed.

Subperiosteal or Perichondrial Development of Bone.—In this form of development the new bone is formed under the *periosteum* by a process like intramembranous. It is the first bone formed, and it continues to be in advance of the intracartilaginous formation.

Growth of Bone.—The long bones grow in length by the ossification extending into the epiphyseal cartilages, which continue to grow at the same time; that part of the shaft already ossified not increasing in length. This is proven by a series of experiments first made by Hales and confirmed by Duhamel and John Hunter. Young animals were taken and the shaft of a long bone exposed. Two holes were bored in the diaphysis and their distance apart carefully measured. After a lapse of considerable time the shaft was again exposed, and it was found that the length of the bone had increased, but the distance between the holes remained the same. If, on the other hand, a hole was bored in the diaphysis and another in the epiphysis, the distance between them, after a certain time, showed an increase.

The shafts of long bones increase in circumference by the new layers of bone formed under the periosteum. At the same time the medullary canal is enlarged by the absorption of the already formed bone from within. This has been proven by the silver ring and madder experiments.

In the first experiment a silver ring is fastened around the circumference of the shaft of a growing bone. This becomes covered with new bone, and the bone which it first surrounded becomes thinner and thinner, is finally all removed by absorption, and the ring becomes free in the now enlarged medullary canal. In the madder experiment, madder root was fed to growing pigs. The external layers of bone formed during this period were found colored red, the lime salts of the bone acting as a mordant for the madder. If the feeding of the madder was stopped for a time, it

was then found that the layers of bone formed during this time remained white and were outside of the red bone, while the white inner layers had become thinner. The *interior* lamellæ of the Haversian systems are colored red while the madder is being fed to the animals. Hunter found that the bone formed at the ends of the diaphysis during the feeding of the madder became colored and showed as a red transverse band, while the bone formed after the feeding of the madder was discontinued was white, thus demonstrating that the formation of bone progressed from the ossification centre toward the ends of the diaphysis.

Regeneration of Bone.—In the repair of fractured bones, bone is formed between and around the broken ends. In some cases the formation of bone is preceded by a formation of cartilage. The periosteum plays an important part in the regeneration of bone. It has been found that if a portion be stripped off, the bone uncovered will die and exfoliate. If a large part of the bone be removed and the periosteum be left intact, the bone will to a large extent be regenerated.

Practical Study.

Intramembranous Development.—Small pieces, including the scalp and dura mater, are taken from the edges of the parietal bone of a new-born child. These are fixed in formalin-Müller's fluid, hardened in alcohol, and decalcified (see page 74). Embed in celloidin and make sections perpendicular to the surface of the scalp. These are stained **double** and mounted in **balsam.**

Low Power.—Observe the irregular-shaped **trabeculæ** of new-formed bone (stained red) lying between the *pericranium* and *dura.*

Make an outline drawing of one of these *trabeculæ* and show its relation to the *dura* and *pericranium.*

High Power.—Select one of the smaller trabeculæ. Observe the row of **osteoblasts** on its outer edge and the layer of **osteogenetic tissue** between them and the pericranium. Note the **bone lacunæ** distributed irregularly in the new-formed bone, and that each contains a **bone cell.** Note the irregular-shaped open-

ings of the trabeculæ, the rows of **osteoblasts** along their edges, and that these openings contain blood vessels and *embryonic* marrow.

Pass to the inner edge of a trabecula and search for an **osteoclast.** Note the pit, **Howship's lacuna,** in which it lies.

Make a drawing of the outer edge of a trabecula showing the *osteoblasts* and their relation to the new-formed bone. Also make a drawing of an *osteoclast* and **Howship's lacuna.**

Intracartilaginous and Subperiosteal Development.—The legs of a fœtal pig of 5 to 6 inches in length are cut off and fixed in formalin-Müller's fluid. After hardening in alcohol they are *decalcified,* embedded in celloidin, and longitudinal sections made. These are stained **double** and mounted in **balsam.**

Low Power.—A. Observe the pair of long bones, surrounded by the **periosteum** and separated from each other by developing muscle, etc.

B. Observe that the upper and lower ends **(epiphyses)** are formed of hyalin cartilage; that the middle portion **(diaphysis)** contains the **ossification zone** and appears as an irregular network.

C. Observe the matrices of the smaller bones composed of hyalin cartilage, and that some of the earlier stages of development *may* show in them.

Make a drawing of one half of one of the *long bones* showing the *epiphysis,* the *diaphysis* and *ossification zone,* and the *periosteum.*

High Power.—A. Note the structure of the **epiphyseal cartilage,** and observe that as the **calcification zone** (stained dark blue) is approached the cartilage cells become arranged in parallel rows; that the cells and cell spaces grow larger; and that finally the cartilage matrix (stained blue) breaks down between the cell spaces, forming irregular cavities, the **Haversian spaces;** that these spaces contain blood vessels and embryonic marrow; and that the cartilage cells have disappeared.

Make a drawing of a portion of the epiphyseal cartilage showing all details.

B. Observe the irregular strings of cartilage matrix (stained blue) extending into the **ossification zone,** forming the partitions

between elongated spaces, the **primitive Haversian canals.** Note the blood vessels and embryonic marrow in these spaces.

C. Observe that the *Haversian spaces* contain, in addition to the elements of embryonic marrow, larger oval cells, the **osteoblasts**, and that these osteoblasts in the commencement of the *primitive Haversian canals* are arranged in rows along the strings of cartilage matrix, and that as the ossification zone is reached a layer of bone (stained red) appears between the osteoblasts and the strings of cartilage matrix; that this layer of bone thickens, and, enclosing the osteoblasts, they become **bone cells**, and the spaces in which they lie, the **lacunæ.** The now complete layer of bones forms a **lamella.** Note that as the original ossification centre is reached, one or more lamellæ of bone have been formed, and that on the edges of the last lamella a row of osteoblasts have formed to produce another lamella.

Make the following drawings:

2. **A.** One showing the *osteoblasts* arranged along one of the cartilage matrix partitions.

B. One showing the commencement of the formation of a bone lamella.

C. One showing a *complete lamella* with its lacunæ and the row of osteoblasts on its edge.

D. Pass to the edge of the ossification zone and study **subperiosteal development.** Observe the **periosteum** and the layer of **osteogenetic tissue** under it. Note that the periosteum becomes continuous with the perichondrium of the epiphyseal cartilage. Observe the irregular-shaped layer of **subperiosteal bone** and that it extends, as a thin layer, up to and even beyond the calcification zone of the epiphyseal cartilage. Note the osteoblasts on the edges of this subperiosteal bone.

Make a drawing showing the process of subperiosteal development.

If the process of bone development has progressed far enough, **osteoclasts** may be found absorbing the new-formed bone.

MUSCULAR TISSUE.

Muscular tissue is divided into three forms: **smooth** or **involuntary, striated** or **voluntary,** and **heart muscle.**

Smooth Muscle

is composed of fusiform, cylindrical, or flattened cells with pointed or branched ends. The cytoplasm is homogeneous and contains a rod-like nucleus. The cellular elements are united to each other by a slight amount of intercellular or cement substance, and are grouped in the form of thin membranes, or fasciculi, which are surrounded by a thin layer of connective tissue, the muscle sheath. A slight amount of connective tissue, in the form of thin septa, penetrates into the interior of the fasciculi. They are often joined by projections of the cytoplasm, the *intercellular bridges.*

Smooth muscle occurs in the gastro-intestinal canal, vascular system, bladder, uterus, and in other organs to a less extent. It is not under the control of the will.

Practical Study.

Smooth Muscle Cells.—Small bits of the muscular coat of the small intestine are placed in a 40 per cent. aqueous solution of potassium hydrate for 3 to 5 minutes; then in a saturated aqueous solution of potassium acetate containing 1 per cent. of hydric acetate for 5 to 10 minutes. Then place the macerated tissue in a test tube nearly filled with water, and shake well in order to isolate the cells. Allow the cells to settle; pour off the water and add alum-carmin for staining the nuclei. After staining 12 hours pour off the staining fluid and wash the cells with water by the

method of decantation. Then place in **eosin-glycerin** for preservation.

High Power.—Select an isolated cell. Observe its shape and the rod-like nucleus.

Make a drawing of a single cell.

Sections of Smooth Muscle Cells.—Small pieces of the small intestine of a cat are fixed in Zenker's fluid, hardened in alcohol, and embedded in celloidin. Transverse sections through the intestine are made, stained **double**, and mounted in **balsam**.

Low Power.—Note the general shape of the section. Observe the ring of smooth muscle surrounding the periphery of the section; that it is divided into two layers, an external, narrow, in which the muscle cells are cut transversely, and an inner, broader layer, in which the cells are cut longitudinally.

High Power.—Select a portion of the outer layer where the cells are cut *transversely*. Observe the shape and size of the sections; that each section is surrounded by a bright, narrow line, the intercellular substance; and that some of the sections contain a *roundish* nucleus.

Make a drawing showing the arrangement of the cells.

Next pass to the inner coat and observe the longitudinal sections of the cells. Note the elongated *rod-like nucleus* and the general arrangement of the cells.

Make a drawing of their appearance.

Striated Muscle.

The **striated muscle fibre** is the morphological equivalent of a cell. It is of a cylindrical shape, and long, measuring from 30 to 120 mm., with a diameter of from 0.1 to 0.01 mm. Their ends are pointed, except where they join tendon, when they are rounded.

The fibres are surrounded by a delicate, homogeneous sheath, the **sarcolemma**, which encloses the **contractile substance.**

The **contractile substance** has two sets of striations: the transverse, which are alternating dark and light bands (discs); and the longitudinal, which show as thin, dark lines running parallel with the long axis of the fibre. Scattered over its surface are a number

of oval-shaped **nuclei** which lie directly under the sarcolemma. Careful examination of the clear disc shows a fine dotted line, **Krause's line**, crossing its central part.

Analysis of the contractile substance shows that it is composed of numerous **fibrils** united by a semi-fluid cement substance, the **sarcoplasm.** They are the contractile part; they extend the entire length of the fibre, and are made up of a series of rod-like masses, the **sarcous elements**, which are placed end to end. These sarcous elements consist of a thicker central portion, which is situated in the dark transverse disc, from each end of which is given off a thin rod which passes into the light disc and terminates in its centre in a bead-like mass. These, extending across the centre of the light disc, form **Krause's line** (Rollett). The muscle fibrils are grouped into small bundles, the **muscle columns** of Kölliker. The sarcoplasm penetrates between the fibrils and muscle columns, being greater in amount between the latter. A muscle fibre in transverse section shows a clear network, the **sarcoplasm,** enclosing dark, polygonal areas, sections of the muscle columns. This appearance is known as **Cohnheim's fields.**

Structure of Muscles.—A **muscle** consists of a collection of **muscle fibres** surrounded by a layer of connective tissue, the muscle sheath or **epimysium.** In the larger muscles the muscle fibres are grouped in bundles, the **muscle fasciculi.** These fasciculi are surrounded by the **perimysium**, prolongations from the epimysium. The muscle fibres of the fasciculi are surrounded by thin layers of connective tissue, the **endomysium.**

Where muscle joins tendon the muscle fibre and its sarcolemma become rounded off, the fibrils of the tendon being attached to the sarcolemma. The blood vessels are numerous and they break up into an elongated capillary network around the muscle fibres.

The nerve supply consists chiefly of medullated nerve fibres which terminate in the neuromuscular end-organs.

Practical Study.

Striated Muscle Fibre.—The structural details of the muscle fibre are best seen when the fibre is in a state of extension. An animal is killed and one of the large muscles of the thigh removed.

This is forcibly extended by fastening one end in a vise and pulling on the other end with the hand. While extended a ½ per cent. aqueous solution of osmic acid is injected into the muscle at various points with a hypodermic syringe, the needle being thrust well into the muscle. After 2 to 3 minutes the fibres become fixed; then pieces are cut out of the browned portion, washed in water, and preserved in *pure* glycerin.

Small bits of the muscle are stained in alum-carmin, placed in a drop of *pure glycerin* on a slide, and teased apart, longitudinally, so as to isolate the fibres, and mounted in the glycerin.

Low Power.—Observe the variation in the diameter of the fibres, and that they are cylindrical in shape. Select an isolated fibre and turn on the

High Power.—Observe the **transverse** markings of the **contractile substance**, caused by the alternating **dark** and **light discs**; the **longitudinal** striations, which indicate that the fibre is made up of **fibrillæ**; pass to the end of the fibre and note the **fibrils** projecting from its broken end. Next observe the shape and distribution of the **nuclei**. Select a light disc and see if **Krause's line** shows. The **sarcolemma** will not show in this specimen.

Make a drawing of a single fibre showing all details of structure.

Transverse and Longitudinal Sections of Muscles.—For this purpose the tongue of a dog is used. This organ is composed of muscles running in all directions, so that sections through it will show the muscles cut in various directions.

The tongue is removed from a recently killed dog and cut, transversely, into slices about half an inch thick. These are fixed in Zenker's fluid, hardened in alcohol, and embedded in celloidin. Sections are cut perpendicular to the dorsal surface and stained as follows: Stain in hæmatoxylin for 20 minutes and wash well in water. Allow the sections to soak in water for at least one hour. Then stain in dilute picroacid fuchsin[1] for 30 seconds, wash in strong alcohol and dehydrate in a second alcohol, clear in oil of origanum, and mount in **balsam.**

Low Power.—Observe the mucous membrane on the dorsal

[1] One per cent. aqueous solution of acid fuchsin, 5 c.c.; saturated aqueous solution of picric acid, 100 c.c.

surface; note the tooth-shaped papillæ and the transverse sections of **muscles** just beneath the mucous membrane.

Select a *transverse* section of a muscle. Observe the transverse sections of the **muscle fibres** grouped in **fascicles**. Note the connective-tissue layer surrounding the muscle, the **muscle sheath** or **epimysium**. Also note that the epimysium sends septa into the muscle, and that these septa surround the fascicles, forming the **perimysium**, and that the muscle fibres are surrounded by a thin layer of connective tissue, the **endomysium**.

Make a drawing of a transverse section of a muscle.

Select a *longitudinal* section of a muscle and make a drawing of it.

High Power.—Select a *transverse* section of **muscle fibre**. Observe the **sarcolemma**, which shows as a thin line surrounding the fibre; note the sections of the **muscle nuclei** embedded in the surface of the **contractile substance**; also note the dotted appearance of the fibre and the bright thin lines, the **sarcoplasm**, which divide the muscle into polygonal-shaped areas, **Cohnheim's fields**. Next note the connective tissue surrounding the fibre, the **endomysium**.

Make a drawing of a single transverse section of a muscle fibre showing all details of structure.

Heart Muscle.

The **contractile substance** of heart muscle has the same structure as striated muscle, but is arranged differently. It occurs in the form of **short, oblong cells**, most of which are branched. They have no **sarcolemma**. Each cell has one, sometimes two, **nuclei**, which are embedded in the cell body. The cell bodies show both a transverse and longitudinal striation, but not so marked as in striated muscle. The heart muscle cells are joined end to end to form muscle fibres, and their branches anastomose with neighboring fibres, forming a narrow meshed net.

Practical Study.

Heart Muscle Cells.—Isolated heart muscle cells are obtained by macerating small bits of fresh heart muscle in the same manner as smooth muscle cells (see page 84).

A drop of glycerin containing the isolated cells is placed on a slide and covered with a cover-glass.

High Power.—Observe the shape of the **muscle cells**; that they give off short, oblique **branches**; that the ends have an irregular appearance; and that each cell contains a **nucleus,** sometimes two. Note that the transverse and longitudinal striations may not show distinctly, and that short pieces of muscle fibre, consisting of two or more cells, may show.

Make a drawing of several cells.

Transverse and Longitudinal Sections of Heart Muscle Fibres.—The papillary muscles in the ventricles, together with a portion of the muscular wall, are cut out. These pieces are fixed in Zenker's fluid, hardened in alcohol, and embedded in celloidin. *Transverse* sections are made through the papillary muscle and the adjoining wall of the heart. These sections are stained **double** and mounted in **balsam.**

High Power.—Observe the *transverse* sections of the heart muscle cells. Note their shape and the variation in size; the shape, size, and situation of the nucleus; the dotted appearance of the sections of cells, which resemble somewhat that of Cohnheim's fields.

Make a drawing showing all details of structure.

Next pass to the *longitudinal* sections of the fibres. Observe their shape; that the fibres lie parallel to each other and give off short, oblique branches which join other fibres. Note the transverse markings and the nucleus.

Make a drawing showing all details of structure.

THE NERVOUS TISSUE.

The nervous tissue is composed of **neurons** and the supporting tissue, the **neuroglia.**

A **neuron** (neuro-dendron, neura, neurocyte) is a nerve cell with all its processes, including the axis-cylinder process or nerve fibre to its termination.

On account of the great length of the nerve fibre it is necessary, for the purpose of study, to divide the neuron into the **nerve cell** and **nerve fibre.**

Nerve Cells.—The nerve cells (ganglion cells) are, as a rule, of an irregular shape. They vary in size, being from 4 to 150 μ in the greatest diameter. A few are spherical in shape, but the vast majority are of a spindle or irregular stellate form. As a rule a nerve cell has two sets of processes, the **protoplasmic processes**, or **dendrites** (dendrons), and the **axis-cylinder process**, or **neuraxon** (Dieter's process, neurite, neuraxone, or axone). Cells having two processes are known as **bipolar**, those having more than two as **multipolar** ganglion cells. Unipolar cells have but one process; they occur chiefly in the ganglia.

The dendrites are thick at their point of origin, they divide repeatedly, and finally terminate in fine fibrils.

The neuraxon (axis-cylinder process) arises from the implantation cone of the nerve cell, in rare instances from the base of one of the dendrites. It is smooth and uniform in its diameter. Sometimes it divides into two equal branches soon after leaving the cell body.

The nerve cells are found in the gray matter of the central nervous system, in the ganglia, and in the sense organs. In certain regions they have fixed and characteristic shapes. In the anterior horns of the spinal cord they are large, multipolar, having numer-

ous dendrites. In the cerebellum we have the large and peculiar-shaped cells of Purkinje with their pear-shaped body, from the lower portion of which is given off the neuraxon and from the upper the dendrites of typical shape and distribution. In the cortex of the brain the cell body is of a pyramidal shape, with a long apex dendrite and numerous smaller ones given off from the sides of the cell body. The neuraxon is given off from the base of the cell or from one of the basal dendrites.

Practical Study.

Nerve Cells of the Human Brain.—Pieces of the convolutions of the human brain are impregnated by the **Golgi mercuric chlorid method.** Pieces about 2 c.c. in size are hardened in formalin-Müller's fluid for at least a month, the fluid being changed frequently. At the end of this time they are placed directly in a ½ per cent. aqueous solution of mercuric chlorid for 15 to 20 days. The mercuric chlorid solution is to be changed daily. The specimens are to be preserved in mercuric chlorid solution, or they may be transferred to 80 per cent. alcohol.

The pieces of tissue that are to be embedded for section-cutting should be thoroughly washed in water and then embedded in the usual manner.

Thick sections are to be cut, 90 μ to 100 μ. They are dehydrated in 97 per cent. alcohol, **cleared in oil of origanum**, and mounted in **balsam.**

This method is not uniform in its results, only portions of the specimen being impregnated, so that the nerve elements will only show in spots.

Low Power.— Select a place in the section where the cells are impregnated. They will show black on a clear ground. Turn on the

High Power.— Observe the cell body, pyramidal in shape; the long apex dendrite with its lateral and terminal branches; the dendrites given off from the sides and base of the cell body; the neuraxon given off, usually, from the base of the cell. Trace the neuraxon as far as possible and see if a collateral branch can be seen.

Note the various irregular-shaped nerve cells and the difference in size of the pyramidal-shaped cells.

Make a drawing of one of the pyramidal nerve cells.

NERVE FIBRES.

The nerve fibres are of two kinds, the **white nerve fibres**, or those **having a medullary sheath**, and the **gray nerve fibres**, or those **without a medullary sheath**.

Non-medullated Nerve Fibres.—These are divided into two groups: those without a neurilemma and those with a neurilemma. Those without a neurilemma consist of the naked axis cylinder (neuraxon of the nerve cell). They are cylindrical in shape, transparent, and grouped in bundles, being held together by a slight amount of connective tissue.

Those with a neurilemma consist of the axis cylinder surrounded by a delicate homogeneous sheath.

Medullated Nerve Fibres.—These medullated nerve fibres are surrounded by a **medullary sheath**, which does not extend their entire length, being absent at their commencement and at their termination.

Some are without a neurilemma, consisting of the axis cylinder surrounded by the medullary sheath, and they occur in the central nervous system alone. Others have both the medullary sheath and neurilemma and are found in the nerve trunks and in the sympathetic nerves.

The **axis cylinder** is the essential part of a nerve fibre. It is the neuraxon of the nerve cell. It is composed of fine **fibrils** cemented together by a semi-fluid substance, the **neuroplasm**, the whole being surrounded by a delicate membrane, the **axolemma** (peri-axial sheath).

The **medullary sheath** is a semi-fluid, fatty substance, the **myelin**, which in the fresh state has a glistening, homogeneous appearance. It is not continuous, but divided at intervals of 80 to 100 μ into the **interannular nodes** by constrictions, the **nodes** or **constrictions of Ranvier**.

The myelin is surrounded, except in the central nervous system, by a delicate, structureless membrane, the **neurilemma**, or

sheath of Schwann. The neurilemma at the constrictions of Ranvier becomes contracted down to the axis cylinder, and at irregular intervals along the interannular node gives off, from its inner surface, thin septa which pass across the myelin and are attached to the axolemma. These septa are the **incisures of Schmidt** or **Lautermann.**

Erwald and Kühne found that upon boiling nerve fibres in alcohol or ether a fine network was brought out in the medullary sheath. This network they named neurokeratin.

Against the inner surface of the neurilemma, about midway between two nodes, are found oval-shaped nuclei, the **nuclei of the neurilemma**, which are surrounded by a small amount of cytoplasm. These nuclei are situated in depressions in the myelin and often cause a slight bulging of the neurilemma.

In the fresh condition the axis cylinder is of a considerable diameter and shows a faint longitudinal striation or may appear homogeneous. After treatment with fixing reagents it shrinks down to a thin cord.

Medullated nerve fibres vary in size. The fine fibres have a diameter of 2-4 μ, the medium-sized 4-9 μ, the large 9-20 μ. The largest number of them are continuous from the nerve cell to the periphery. A few branch, and this branching always takes place at a node of Ranvier.

Practical Study.

Fresh Nerve Fibre.—A frog is killed and the abdominal cavity opened and the lumbar nerves exposed. A small bit of one of the nerves is cut out with scissors and placed on a slide with a drop of sodium chlorid solution. This is teased apart, longitudinally, with the points of the teasing needles. Then put on a cover-glass.

Low Power.—Select a place in the specimen where the nerve fibres are isolated and turn on the

High Power.—Observe the **axis cylinder** in the centre of the fibre, having a homogeneous or very faintly striated appearance. Note its diameter and that it is bounded by a double contour, the **medullary sheath**, the bright yellowish substance between the parallel lines being the **myelin.**

The action of the salt solution soon causes changes in the myelin. The myelin, swelling, causes the fibre to assume an irregular shape, and at times it may be seen pouring out of the broken ends of the fibre.

Make a drawing of a single nerve fibre showing its appearance before and after the changes in the myelin.

Human Nerve Fibre.—Several nerves are removed from the human cauda equina and suspended in a large quantity of formalin-Müller's fluid for hardening. This will require from four to five weeks. They are then washed well in water and preserved in 80 per cent. alcohol. A piece of the nerve, half an inch long, is teased apart, *longitudinally,* into thin strands, and these are stained in picro-acid fuchsin. This stain is prepared as follows: 1 per cent. of acid fuchsin, 15 c.c., and saturated aqueous solution of picric acid, 50 c.c., are mixed with 50 c.c. of water. The thin strands of the nerve are placed in this stain for fifteen to twenty minutes; they are then washed in 97 per cent. alcohol until the red color ceases to come away. Then they are placed in pure oil of origanum for clearing.

One of the strands is placed on a slide with a small quantity of the oil and carefully teased apart, longitudinally, with the teasing needles, in order to isolate a number of nerve fibres.

After teasing, absorb the excess of oil with bits of filter paper, put on a drop of balsam and then a cover-glass.

Low Power.—On account of the thickness of the strand of the nerve fibres, the peripheral fibres are most likely to be overstained, the central fibres under-stained, while a zone of fibres between the two will be stained properly.

Select an isolated and properly stained fibre and turn on the

High Power.—Observe the **axis cylinder,** shrunken down to a thin cord (stained red) in the centre of the fibre; trace it across a **node** or **constriction of Ranvier;** the **neurilemma** (stained red) and its septa, the **incisures of Schmidt** (stained red), passing across the medullary sheath; the **nucleus** and its surrounding cytoplasm (stained red); and the myelin (stained yellow) between the neurilemma and the axis cylinder.

Make a detailed drawing of an **interannular node.**

THE NERVE TRUNKS (NERVES).

The nerves are composed of bundles of nerve fibres, the **fascicles** (funiculi), which are surrounded by a laminated sheath, the **lamellar sheath** (perineurium). The fascicles (funiculi) are joined to each other by connective tissue, the **perifascicular connective tissue** (epineurium), which also forms a sheath for the entire nerve. The lamellar sheath sends septa into the interior of the fascicles, the **intrafascicular connective tissue** (endoneurium). These septa divide the fascicle into compartments of different sizes and they contain the nerve fibres.

The lamellar sheath is composed of several layers of fibrillated and elastic fibres, and the spaces between the layers are lined with endothelial cells, lymph spaces.

Blood vessels are found in both the intrafascicular and perifascicular connective tissue.

Practical Study.

A small nerve of the size of the human radial or ulnar is suspended in formalin-Müller's fluid and *hardened*. Small pieces are embedded in celloidin and transverse sections made.

The sections are stained as follows: Stain in hæmatoxylin for half an hour and then remove and wash well in water. Allow the stained sections to soak in water over night. Then stain in picro-acid fuchsin (for formula see nerve fibre, page 94) for one-half to three-quarters of a minute; then wash in two alcohols (97 per cent.), clear in oil of origanum, and mount in **balsam**.

Low Power.—Observe the number, size, and shape of the **fascicles** (funiculi); the **lamellar sheath** (perineurium) and its septa; the **intrafascicular connective tissue** (endoneurium) dividing the interior of the fascicle into compartments; the transverse sections of the **nerve fibres**; and the **perifascicular connective tissue** (epineurium) binding the fascicles to each other. Note the blood vessels in the intrafascicular and perifascicular connective tissue.

Make an outline drawing of several fascicles and show their relation to each other.

High Power.—Observe the structure of the **lamellar sheath;** the **intrafascicular connective tissue;** and the **neuroglia** surrounding the nerve fibres. Note the transverse sections of the nerve fibres; their different diameters; their axis cylinders (stained red) in the centre of the surrounding myelin of the medullary sheath (stained yellow). See if the neurilemma can be made out.

Make a drawing of several nerve fibres showing all details of structure.

THE VASCULAR SYSTEM.

THE HEART.

The heart has three coats: the internal, or **endocardium**; the middle, or **myocardium**, or muscular layer; and the external, or **pericardium**.

The **endocardium** is composed of a single layer of endothelial cells which lie on a thin layer of smooth muscle and connective tissue containing numerous elastic fibres.

The **myocardium** is made up of heart muscle tissue (see page 88) laid down in several layers. In the auricles two layers are found and in the ventricles three. All the muscular layers as well as the muscle bundles are supported by connective tissue containing many capillaries.

The **pericardium** consists of two layers: the **visceral** (epicardium), which is closely adherent to the myocardium, and the **parietal**, which forms the external wall of a closed sac, the cavity of the pericardium. This layer is continuous with the visceral layer at the base of the heart. The pericardium consists of a connective-tissue stroma, the free surface of which is covered with a single layer of mesothelial cells.

The valves of the heart are duplications of the endocardium and have essentially the same structure.

THE BLOOD VESSELS.

The blood vessels are of three kinds, **arteries**, **veins**, and **capillaries**. The capillaries establish the communication between the arteries and veins.

The **capillaries** are tubes the walls of which are made up of a single layer of endothelial cells. They anastomose with each other, forming a network, the meshes of which vary in size and shape in different regions.

The **arteries** are classified as follows: **small** or **arterioles, medium-sized**, and **large**. Their walls are made up of three coats, the **intima**, the **media** or **muscular**, and the **external** or **adventitia** composed of connective-tissue elements.

A **small artery**, or **arteriole**, has an intima consisting of a single layer of endothelial cells. The media is formed of a single layer of smooth-muscle cells supported by a small amount of connective tissue. The external layer, or adventitia, consists of a small amount of connective tissue.

A **medium-sized artery** has the same three coats, but these have certain structural differences. The intima is composed of three layers, the internal lining of **endothelium**. External to this is a connective-tissue layer, the **intermediary layer** of the intima or **subendothelial** layer, composed of fine, fibrillated and elastic fibres and flattened connective-tissue cells. The third layer, or **elastic layer**, of the intima is a thin, fenestrated membrane of elastic tissue closely connected with the media and marks the external limits of the intima. The **media** is a broad layer of smooth-muscle tissue, circularly arranged, the muscle bundles being supported by a small amount of connective tissue. The adventitia is composed of fibrillated and elastic fibres loosely arranged.

The majority of the arteries belong to this class, ranging in diameter from that of brachial to the supraorbital.

The **large arteries** are the aorta, carotids, pulmonary, etc. They have the three coats to their walls, but the latter are comparatively thin. This thinness is compensated for by the increase in strength of the media.

In a large artery, like the aorta, the intima has the internal lining of endothelial cells and the intermediary layer of fibrillated and elastic fibres and cells. As the outer portion of this layer is reached the elastic fibres become converted into a fenestrated membrane corresponding to the elastic layer of the intima of the medium-sized arteries, but not so sharply defined. In the media the elastic tissue preponderates over the other tissues. It is arranged in a series of circular lamellæ of fenestrated membranes, between which are smooth-muscle cells and connective-tissue fibres. The adventitia is composed of fibrillated and elastic

fibres, loosely arranged and running longitudinally. It also contains blood vessels, the vasa vasorum.

The walls of the **veins** have, like the arteries, three layers, made up of the same elements, but not laid down in so distinct a manner. In a vein corresponding in size to a medium-sized artery there are three layers, the intima, media, and adventitia. The intima consists of a single layer of endothelial cells, a thin connective-tissue layer, the intermediary layer, and an ill-defined elastic lamina. The media, much less thick than that of a corresponding sized artery, consists of bundles of smooth muscle arranged in a circular manner, sometimes being continuous, but more often broken up into isolated bundles which are surrounded by considerable connective tissue. The adventitia has the same structure as that of an artery of the same size, except that it possesses a well-developed longitudinal layer of smooth muscle on its inner edge.

In the **smaller veins** the intima consists of a single layer of endothelial cells; the media, chiefly of connective-tissue elements with a few scattered bands of smooth muscle; the adventitia is thin and may contain a few smooth-muscle cells. The precapillary veins, those connecting directly with the capillaries, are thin-walled, the intima having disappeared.

The **valves** of the veins are folds of the intima projecting into the lumen of the veins. They have the same structure as the intima. They are semilunar in shape and generally consist of two folds placed opposite to each other. The convex border is attached to the side of the vein, the other edge being free and pointing toward the heart.

LYMPH VESSELS.

The large lymph vessels have a structure essentially the same as veins and have valves. The larger vessels become smaller and pass over into the **lymph capillaries**, which in turn are connected with the cell spaces of the tissues. The lymph capillaries are formed of a single layer of endothelial cells; they are of a greater diameter and more irregular in shape than the blood capillaries. The blood vessels are surrounded by narrow spaces lined with en-

dothelium, the **perivascular spaces,** which are in connection with the lymph vessels. In other portions of the body we have similar spaces lined with endothelium, the **perilymphatic spaces.** The subdural spaces of the pia, the subarachnoidal space, the lymph sinuses, and the perilymphatic spaces of the ear are examples.

Practical Study.

30 **Capillaries, Arterioles, and Small Veins.**—Slices of the surface of the brain, about an inch thick, are hardened in formalin-Müller's fluid, and the portions of the pia dipping down into the sulci between the convolutions are removed, washed in water, and preserved in 80 per cent. alcohol. Small bits, a quarter of an inch square, are stained **double,** cleared, spread out flat on a slide, and mounted in **balsam.**

Low Power.—Select a thin place in the specimen and observe the **capillary network.** Some of the capillaries may be distended with blood, others may be collapsed. See if a capillary can be traced into a vein.

High Power.—A. (1) Select a capillary distended with blood. Observe its walls, showing as parallel thin lines with oval-shaped nuclei projecting into the mass of blood cells. (2) Select a collapsed capillary. Observe its appearance. (3) Observe the capillary network.

Make a drawing of the capillary network; of a distended capillary; and of a collapsed capillary.

B. Select a small vein and observe the structure of its walls, and make a drawing of it.

C. With the low power select an arteriole and turn on the

High Power.—Focus on the convex surface of the arteriole. Observe the rod-like nuclei of the smooth-muscle cells, running nearly transversely; the oval-shaped nuclei of the endothelial cells of the intima, running longitudinally.

Make a drawing of this appearance.

Next make an *optical longitudinal section* of the arteriole. This is accomplished by focussing down through its convex surface until the walls of the arteriole show as if they had been cut longitudinally.

Observe the two parallel walls of the arteriole. Note the intima, composed of a single layer of endothelial cells; the media, a single layer of smooth-muscle cells, appearing as if they had been cut transversely; the adventitia, connective-tissue elements.

Make a drawing showing the three coats.

Endothelium of Blood Vessels.—The outlines of the endothelial cells of blood vessels are brought out by silver impregnation. This is accomplished by washing out the entire vascular system of a small animal with water, as follows: Introduce a glass canula into the thoracic aorta and inject water; then inject a 1 per cent. aqueous solution of silver nitrate; cut out some of the smaller vessels, slit them up lengthwise, and lay them out flat on a slide, inner surface uppermost. Put on a drop of glycerin and expose the specimen to the sunlight; when it has become brown put on a cover-glass and examine with the high power. The outlines of the cells will be marked out by fine black lines.

The endothelial cells of the capillaries are best demonstrated in the bladder of the frog after the method described on page 57.

Sections of Medium-Sized Arteries and Veins.—An artery corresponding in size to the radial artery, with its accompanying vein, is carefully dissected out and fixed in formalin-Müller's fluid by suspending it, with a small weight attached to one end, in a tall vessel containing the fixing fluid. Small pieces, half an inch long, are embedded in celloidin and transverse sections made. These are stained **double** and mounted in **balsam.**

Low Power.—Bring a portion of the walls of the artery and vein into the field of the microscope. Compare the thickness of their walls and note the arrangement and thickness of the different layers.

Make a drawing of the artery and of the vein showing the three layers of each.

High Power.—1. Select the wall of the artery. Observe the layers of the **intima,** its **endothelial layer,** its **intermediary** or **subendothelial layer,** and its **elastic lamina,** more or less folded. Next observe the structure of the **media** and, finally, the **adventitia.**

Make a drawing showing the details of structure of each layer.

2. Select the wall of the vein and proceed as in 1.

3. Select a transverse section of an arteriole and proceed as in 1.

Section of the Aorta.—Portions of the aorta are removed, slit up longitudinally, and pinned out on a piece of sheet cork. They are fixed in formalin-Müller's fluid, hardened in alcohol, and embedded in celloidin. Sections are made perpendicular to the surface, stained **double,** and mounted in **balsam.**

Low Power.—Observe the three layers of the wall and make an outline drawing showing their proportions.

High Power.—Observe the structure of the intima; the media, with its elastic tissue; and the adventitia, with the **vasa vasorum.**

Make a drawing of a section through the entire wall of the aorta, showing all details of structure.

Elastic Tissue of the Aorta.—Sections of the aorta, fixed as above, are stained in **Weigert's elastic fibre stain.** This stain is prepared as follows: fuchsin, 2 gms.; resorcin, 4 gms.; water, 200 c.c. Boil in a porcelain dish, and while boiling stir in 25 c.c. of liquor ferri sesquichlorati and continue the boiling for five minutes. A precipitate forms. After cooling, filter. When the precipitate has become dry, place it and the filter paper in a porcelain dish and add 200 c.c. of 95 per cent. alcohol. Boil until the precipitate is dissolved, cool, filter, and make up the filtrate to 200 c.c. by the addition of 95 per cent. alcohol. Then add 4 c.c. of hydric chlorid.

The sections are stained in this fluid for half an hour; then washed in 97 per cent. alcohol until all color ceases to come away; clear in oil of origanum and mount in **balsam.**

High Power.—Observe the amount, thickness, and arrangement of the elastic fibres (stained bluish) and make a drawing of them.

LYMPHATIC TISSUE AND LYMPHATIC ORGANS.

Structure of Lymphatic Tissue.—Lymphatic tissue is composed of a basement substance of **reticular connective tissue** in the meshes of which are packed the **lymph cells.**

The **reticular connective tissue** is composed of delicate fibrillated connective-tissue fibres which form an irregular-meshed network having thickened points, the nodal points, at the junction of several fibres. The entire reticulum is covered with a single layer of flat cells.

The **lymph cells** are of a spherical shape, with a large, spherical-shaped nucleus, the cytoplasm being exceedingly small in amount. They resemble the lymphocytes of the blood.

Lymphatic tissue occurs as **diffuse** or **circumscribed.** The diffuse form is found in some of the mucous membranes; the circumscribed, as **nodules** in various organs.

The **lymphatic organs** are the lymph nodes, tonsils, thymus body, and spleen.

THE LYMPH NODES.

The lymph nodes, erroneously called lymph glands, are rounded, oval, flat, or kidney-shaped bodies occurring in the course of the lymph vessels of the body.

A **lymph node** is surrounded by a connective-tissue **capsule,** and at one side there is a slight depression, the **hilus,** where the arteries enter and the veins and efferent lymph vessels emerge. The afferent vessels penetrate the capsule at various points.

The **capsule** sends into the interior of the node **septa** or **trabeculæ** which divide the **cortex** into a series of irregular-shaped compartments; finer branches of the trabeculæ pass on to the **medulla,** forming a series of irregular communicating channels somewhat tube-like in shape. The capsule and trabeculæ contain a slight amount of smooth muscle.

The **lymphatic tissue** in the lymph node occurs in two forms, **lymph nodules** and **lymph cords** (medullary cords).

The **lymph nodules** occupy the compartments of the cortex. They are oval or spherical in shape, and at the edge of the medulla break up into cord-like masses, the **lymph cords**. The lymph cords anastomose with each other and form a network which occupies the channels in the medulla formed by the finer trabeculæ. The lymph nodules are absent at the hilus.

The central portion of the nodules often shows a roundish zone where the lymph cells are not so densely packed. This is the **germ centre** of Flemming, and it is here that the proliferation of the cells takes place. They are not permanent structures, being absent for a time.

The lymph nodules and lymph cords are surrounded by narrow channels, the **lymph sinuses**. These sinuses are bridged across by reticular connective tissue which is attached to the trabeculæ on one side and becomes continuous with the reticulum of nodules or cords on the other.

The **afferent lymph vessels** pierce the capsule, generally opposite to the hilus, and open into the lymph sinuses of the cortex. The lymph is poured into these and flows through these and those of the medulla toward the hilus, where it is taken up by the **efferent vessel** or **vessels.**

The arteries enter the node through the hilus; entering the trabeculæ they pass into the interior of the organ, breaking up into capillaries in the lymph nodules and lymph cords. The arterial capillaries pass into the venous; these, uniting, form the veins which pass out of the organ alongside of the arteries.

Practical Study.

(1) **Sections of Lymph Node.**—The chain of the cervical lymph nodes are exposed in a recently killed dog. A small canula is introduced into the substance of the uppermost node and formalin-Müller's fluid slowly injected into it. When the node has become tense the pressure is increased slightly, in order to force the fluid into the other nodes of the chain. As soon as all have become tense the canula is withdrawn and the nodes carefully dissected

out and placed in formalin-Müller's fluid for twenty-four hours; then washed in water and hardened in alcohol. After embedding in celloidin longitudinal sections are made, stained **double,** and mounted in **balsam.**

Low Power.—1. Observe the **capsule** and its **trabeculæ** extending into the interior of the node, and that they divide the cortex into compartments and the medulla into irregular passages. Note that in the medulla transverse or oblique sections of the trabeculæ are the most numerous.

2. Observe that the compartments of the cortex are nearly filled with large masses of lymphatic tissue, the **lymph nodules.** Note that the nodules break up into cord-like masses, the **lymph cords,** which extend into the medulla, forming a network of lymphatic tissue. Also note that at the hilus the lymph nodules are absent.

3. Observe the narrow channels surrounding all of the lymphatic tissue, the **lymph sinuses.**

Make a drawing of two or more lymph nodules and their cords, showing their relation to the capsule, trabeculæ, and lymph sinuses.

High Power.—1. Observe the structure of the lymph nodules and lymph cords. Note that they are made up of spherical-shaped cells with large nuclei, the **lymph cells.**

2. See if any of the lymph nodules have the **germ centre.**

3. Observe the structure of the **capsule** and **trabeculæ,** and that the sections of many of the latter contain blood vessels.

4. Select a lymph sinus as free from cells as possible and note the **reticular connective tissue** stretching across it. Note that this reticulum is attached to the trabeculæ and the edges of the lymph nodules and lymph cords.

5. Select a lymph cord in which most of the lymph cells have been washed out in the preparation of the specimen, and observe the framework of reticular connective tissue in the meshes of which a few lymph cells remain.

Make a drawing showing the appearance of the lymphatic tissue and of the reticular connective tissue.

THE TONSILS.

The pharyngeal tonsils are composed of lymph nodules em-

bedded in diffuse lymphatic tissue. Their free surface is covered with stratified squamous epithelium, which also lines the crypts or recesses extending into the organ. The diffuse lymphatic tissue surrounds these crypts, and in it are embedded a row of lymph nodules. The epithelium of the crypts opposite the lymph nodules shows degenerative changes. The epithelial cells are absorbed and their places are taken by lymph cells. This is the so-called lymphoid infiltration.

Practical Study.

Tonsil of Dog.—The pharyngeal tonsils of a dog are removed and fixed in formalin-Müller's fluid, hardened in alcohol, and embedded in celloidin. Sections are cut perpendicular to the surface of the tonsil, so as to include a crypt. They are stained **double** and mounted in **balsam.**

Low Power.—Observe the surface of the tonsil, and note that it is covered with stratified squamous epithelium, which also lines the interior of the **crypts.** Note the row of **lymph nodules** embedded in the **diffuse lymphatic tissue,** and that they are arranged around the crypt.

Make an outline drawing showing a crypt, its lining epithelium, and the position of the lymph nodules.

High Power.—Observe the lymphatic infiltration of the epithelium of the crypt, and make a drawing showing its details.

THYMUS GLAND.

The thymus gland is composed of lobes which are made up of lobules varying in size. The lobules are separated from each other by connective tissue in which there are blood and lymph vessels. The lobule is divided into the cortex and medulla. The cortex is divided into compartments by short septa of connective tissue. The lymphatic tissue is in the form of nodules in the cortex and of a diffuse form in the medulla. The medulla contains peculiar epithelial structures, **Hassal's corpuscles,** composed of flattened cells arranged concentrically around a central core of degenerated cells. They are believed to be the remains of the epithe-

lium of which the gland was originally composed. The gland is abundantly supplied with lymph and blood vessels.

Practical Study.

Thymus Gland of Child.—The thymus gland of a new-born child is fixed in formalin-Müller's fluid, hardened in alcohol, and embedded in celloidin. Sections are stained **double** and mounted in **balsam.**

Low Power.—Select a small **lobule.** Observe the connective tissue surrounding it and its septa passing into the cortex. Note the lymph nodules in the cortex, and the medulla containing Hassal's corpuscles and large blood vessels.

Make a drawing of a single lobule.

High Power.—Note the structure of the nodules and the medulla; also of Hassal's corpuscle.

Make a drawing of a Hassal's corpuscle and its surrounding tissue.

THE SPLEEN.

The spleen is surrounded by a **capsule** of connective tissue containing a few smooth-muscle cells, its external surface being covered by the peritoneum. This capsule sends **trabeculæ** into the organ, which break up into branches, and these, uniting with branches of trabeculæ that pass in at the hilus with the blood vessel, form a framework for the support of the spleen tissue. Scattered through the organ are oval-shaped masses of lymphatic tissue, the **lymph nodules** or **Malpighian bodies.** These surround small arteries and are embedded in the spleen pulp. They have the same structure as the lymph nodules of the lymph node.

The **splenic artery** enters the spleen at the hilus and divides into two sets of branches. One set pass into the trabeculæ, the other into the spleen pulp, furnishing the arteries of the lymph nodules. Their terminal branches pass into the pulp cords, where they end in dilatations, the **ampullæ.** The walls of the ampullæ as they pass over into the veins, as well as the commencement of the veins, are fenestrated.

The **veins** commence in the spleen pulp as irregular communicating channels, the **cavernous veins**; these, uniting, form veins which empty into those found in the trabeculæ; the latter, uniting, form the **splenic vein,** which passes out of the organ at the hilus.

The **pulp cords** surround the **cavernous veins** and are composed of a reticulum the meshes of which are filled with a variety of cells—red blood cells; nucleated red blood cells; large cells containing red blood cells, fragments of red blood cells, and pigment; and the various forms of white blood cells. In isolated preparations of the cells long, spindle-shaped cells with bulging nuclei, endothelial cells from the ampullæ, are also found.

According to Mall,[1] the spleen is composed of lobules, small spleens having 25,000, large 200,000, with an average of 80,000. The lobule is about 1 mm. in diameter and is bounded by three interlobular trabeculæ, each sending three intralobular trabeculæ into the lobule, which, branching, divide the lobule into ten compartments. The artery enters at one end of the lobule, branches and sends a branch to each compartment, where it terminates in the pulp cords in dilatations of the ampullæ. The spleen pulp fills these compartments and is arranged in cord-like masses, the pulp cords. These surround the intralobular venous spaces (cavernous veins), which communicate with the intralobular veins and these with the interlobular veins found in the trabeculæ.

Practical Study.

Cat's Spleen for Topography.—The spleen of a cat is fixed in formalin-Müller's fluid, hardened in alcohol, and embedded in celloidin. Transverse sections are made through the entire spleen. The sections are stained **double** and mounted in **balsam.**

Low Power.—1. Observe the **capsule** surrounding the section; note that it sends **trabeculæ** into the interior, which branch and form a framework.

2. Observe the masses of lymphatic tissue, the **lymph nodules** or Malpighian bodies. Note the section of the artery.

[1] Bulletin of the Johns Hopkins Hospital, ix., 1898, p. 218.

3. Observe that the lymph nodules are surrounded by tissue containing many cells, the **spleen pulp.**

Make a drawing showing the capsule, the trabeculæ, the lymph nodules with the artery, and the spleen pulp.

Human Spleen Congested.—Pieces of a congested human spleen are fixed in formalin-Müller's fluid, hardened in alcohol, and embedded in celloidin. Sections are stained **double** and mounted in **balsam.**

Low Power.—Observe the cavernous veins filled with blood, and that they are surrounded by cord-like masses of cells, the pulp cords.

Make an outline drawing showing the relations of the cavernous veins to the pulp cords.

Human Spleen.—Small pieces of the human spleen are fixed in Zenker's fluid, hardened in alcohol, and embedded in celloidin. Sections are stained **double** and mounted in **balsam.**

Low Power.—Observe the **capsule, trabeculæ, lymph nodules,** and **spleen pulp.** Note the cavernous veins, that the most are collapsed and appear as irregular slit-like openings.

High Power.—1. Observe the structure of the **capsule** and **trabeculæ.** Make a drawing showing these structures.

2. Observe the structure of the **lymph nodule.** Make a drawing of a portion of the nodule with its artery.

3. Observe the structure of the **pulp cords** and **cavernous veins.** Make a drawing of a vein and its surrounding pulp cords.

Isolated Cells of the Spleen.—An incision is made into a fresh human spleen and the surface of the cut scraped with a knife. The scrapings are fixed in Zenker's fluid, hardened in alcohol, and stained in alum-carmin and then preserved in **eosin-glycerin.**

A drop of the eosin-glycerin containing the cells is placed on a slide and a cover-glass put on.

High Power.—Identify the different cells and make a drawing of each variety.

SEROUS MEMBRANES, MUCOUS MEMBRANES, AND GLANDS.

SEROUS MEMBRANES.

The serous membranes are composed of a thin layer of connective tissue with a network of elastic fibres at its inner edge. Their free surface is covered with a single layer of mesothelial cells. Some of the serous membranes have openings, the stomata, which communicate with the underlying lymphatics. The stomata are surrounded by a single layer of cuboidal-shaped cells.

Serous membranes are closed sacs, one wall of which is attached to the inner surface of the cavity which they line, and is known as the *parietal* layer; the other covers the surface of the organ or organs and is known as the *visceral* layer. There is one exception, the female peritoneum, which has two openings by which the lumen of the Fallopian tubes communicates with its cavity.

MUCOUS MEMBRANES.

The mucous membranes line cavities which communicate with the exterior. They are composed of a **stroma** (corium, tunica propria); **epithelium**, which covers the surface; a thin layer of smooth muscle, the **muscularis mucosæ;** and the **submucosa,** a connective-tissue layer. The most of the mucous membranes also contain **glands.**

The stroma is composed of connective tissue. Its surface next the epithelium is covered by a membrana propria which is not always demonstrable and is often incomplete.

The epithelium varies in type in different regions. It may be stratified squamous, transitional, simple cylindrical, or stratified cylindrical. The cylindrical type in some instances is ciliated.

The muscularis mucosæ is composed of smooth muscle. It

forms the external limit of the mucous membrane, but it is not always present.

The submucosa is composed of moderately coarse connective-tissue fibres loose in arrangement. It contains the larger trunks of the blood vessels, and lymphatics.

The glands of the mucous membranes are of the simple or compound tubular form.

GLANDS.

Glands are composed of epithelium which rests on a structureless membrane, the membrana propria. In a few instances the membrana propria is composed of flat cells laid down in layers. Not all of the epithelium lining a gland secretes, hence the division into duct and secretory portions.

All glands are abundantly supplied with blood vessels and nerves.

Glands are classified, according to their shape, into:

1. Tubular glands:
   - *(a)* Simple.
   - *(b)* Compound.
2. Saccular:
   - *(a)* Simple.
   - *(b)* Compound.

A simple tubular gland is a tube-like structure, uniform in diameter. This type of gland may divide into branches, or its deeper portion may become more or less coiled.

A compound tubular gland consists of two or more secretory tubules which empty into one of a series of excretory ducts.

A simple saccular gland consists of a single sac-like expansion connected with a short excretory duct.

A compound saccular gland consists of sacs or alveoli grouped around terminal ducts.

All compound glands of any size are divided into **lobes** and these into **lobules**. The entire gland is surrounded by a connective-tissue **capsule** which sends septa into its interior. These septa separate the lobes and lobules from each other. Compound glands have a main or **excretory duct**; this branches and sends

at least one branch to each lobe, the **lobar duct.** The lobar duct, dividing, sends branches to the lobules, which divide and form the **terminal ducts,** around which are grouped the secretory portion of the gland, the **alveoli.** The ducts have a membrana propria, upon which rests a single layer of cylindrical epithelium. In the large ducts the epithelium is high; as the ducts grow smaller it decreases in height, and in the terminal ducts it becomes flat.

The microscopic appearance of gland cells varies according as they are in a state of activity or rest. The cytoplasm of active cells is increased in volume and has a clear appearance, while resting cells show a decrease in volume and have a dark appearance.

Some gland cells discharge their secretion from their sides as well as from their free surface. In such cases the cells are surrounded by a network of canaliculi, the *secretory capillaries;* these, uniting, form a canal which communicates with the lumen of the gland.

In the process of the formation of the secretion the cells, in some glands, become destroyed, their places being taken by new cells. In other cases only a portion of the cell becomes destroyed and the cell becomes regenerated from the remaining cytoplasm which contains the nucleus.

THE DIGESTIVE ORGANS.

The digestive organs consist of the gastro-intestinal canal and the various glands whose secretions are discharged into it by ducts. The gastro-intestinal canal commences at the mouth and terminates at the anus. It is divided into various divisions. The mouth, pharynx, and œsophagus lie above the diaphragm; the stomach, small and large intestines in the abdominal cavity or cavity of the peritoneum; the rectum in the pelvic cavity. The large glands that empty their secretion into the canal are the salivary glands, pancreas, and liver.

THE MOUTH.

The mouth, oral cavity or buccal cavity, is lined with mucous membrane the free surface of which is covered with stratified squamous epithelium resting on a stroma or tunica propria, which is thrown up into numerous papillæ. This stroma is made up of fibrillated connective-tissue fibres with a few elastic fibres and considerable diffuse lymphatic tissue with an occasional lymph nodule. It also contains numerous glands of the branched tubular variety. The stroma gradually merges into a loose connective-tissue layer, the submucosa.

THE TONGUE.

The tongue is made up of voluntary muscles which run in various directions. It is covered by a mucous membrane similar in structure to that lining the mouth, except that the papillæ on its upper surface are of a more complicated structure. These papillæ are of three kinds: the **filiform,** the **fungiform,** and the **circumvallate.**

The filiform papillæ are the most numerous. They consist of a main papilla of connective tissue, which is long and thin, from the

summit of which several secondary papillæ are given off. They are covered by stratified squamous epithelium which over the secondary papillæ becomes hornified and projects as filamentous processes.

The fungiform papillæ are comparatively few in number and are distributed in an irregular manner between the former. They consist of a main papilla, the summit of which is rounded, the base being somewhat constricted. Secondary papillæ are given off from its summit and sides. The epithelium covering them is somewhat thin and is not hornified.

The circumvallate papillæ are confined to the back portion of the upper surface of the tongue. They are from eight to fifteen in number and are arranged in two rows, nearly at right angles to each other, with the apex pointing backward. They are deeply embedded in the mucous membrane, the latter forming a wall around their sides. They consist of a broad main papilla of connective tissue with a flattened or slightly convex summit, from which are given off short secondary papillæ. Their surface and sides are covered with stratified squamous epithelium. In the epithelium covering the sides are found the **taste-buds.** These are oval-shaped masses of flattened cells. These cells are of two varieties, the sustentacular and the neuro-epithelial cells.

The mucous membrane of the root of the tongue contains two kinds of branched tubular glands. One kind secretes mucus and are called mucous glands; the second kind secretes a serous fluid and are called serous glands.

The **lymph follicles** of the tongue are situated in the mucous membrane between the circumvallate papillæ and the epiglottis. They are collections of lymph nodules embedded in diffuse lymphatic tissue. In the centre of the follicle there is an opening, the crypt, which is lined with stratified epithelium, a continuation of the surface epithelium. These follicles are sometimes called the **lingual tonsils.**

THE ŒSOPHAGUS.

The walls of the œsophagus consist of four layers: the **external** or **fibrous,** the **muscular,** the **submucosa,** and the **mucous membrane.**

The fibrous coat is composed of connective-tissue fibres arranged in compact bundles interspersed with numerous and rather coarse elastic fibres.

The muscular coat is made up of two layers, an **external longitudinal** and an **internal circular**. In the upper portion the muscular tissue is of the *striated* variety; at about the middle portion it is a mixture of both *striated* and *smooth muscle;* in the lower portion it is composed of *smooth muscle* entirely. The two layers are separated by a thin band of connective tissue which sends in septa between the muscle bundles.

The submucosa is composed of loose connective tissue and contains the larger blood vessels, lymphatics, and nerves. In the upper portion of the œsophagus this coat contains mucous glands, the ducts of which pass through the mucosa and open on the free surface of the mucous membrane. Similar glands also occur in the lower end.

The mucous membrane has a well-developed **muscularis mucosæ,** which is composed of a longitudinal layer of smooth muscle. Internal to this is the **stroma,** the surface of which is thrown up into conical-shaped **papillæ.** The free surface is covered with a layer of stratified squamous epithelium.

GENERAL STRUCTURE OF THE STOMACH AND INTESTINES.

The walls of the stomach and intestines are composed of four coats: the **serous,** the **muscular,** the **submucosa,** and the **mucosa** or **mucous membrane.**

The serous coat is the external one and is a fold of the peritoneum. It covers the entire surface except at the attachment of the mesentery. The connective-tissue layer, the subserosa, is covered externally by a single layer of mesothelial cells.

The muscular coat is composed of smooth muscle and is divided into two layers, the outer or **longitudinal** and the inner or **circular.** The two layers are separated by a thin band of connective tissue, which sends prolongations into both muscular layers for the support of the muscle bundles. At the cardiac end of the

stomach an incomplete oblique layer is found internal to the circular layer.

The submucosa is of considerable thickness and is composed of areolar connective tissue. Being loose in structure, it permits the mucous membrane to move freely over the muscular layer, and the former, becoming thrown up into folds, forms the **valvulæ conniventes** of the small intestine and the **rugæ** of the stomach. It contains the larger trunks of the blood vessels, lymphatics, and nerves.

The mucous membrane consists of a **muscularis mucosæ, stroma, glands,** and **surface epithelium.** The muscularis mucosæ is made up of two layers of smooth muscle, an external longitudinal and an internal circular. The stroma is composed of connective tissue moderately rich in cells. In some portions of the intestine it assumes the characteristics of lymphatic tissue. The glands are of the tubular type in the intestine and of the branched tubular form in the stomach. The surface epithelium is of the simple cylindrical type.

The blood supply is abundant. The arteries enter at the attachment of the mesentery, pass through the muscular layers, giving off branches, and upon reaching the submucosa form a network of large vessels (Heller's plexus). From these branches are given off. One set of these supplies the muscularis mucosæ; the other passes through the muscularis mucosæ and breaks up into capillaries which surround the glands of the mucosa and terminate in the subepithelial plexus just beneath the surface epithelium. The veins arise from this plexus and, passing through the mucosa between the glands, form a plexus internal to the muscularis mucosæ; from this small veins pass through the muscularis mucosæ and empty into the venous plexus of the submucosa, the branches of which, uniting, form larger veins which empty into the larger venous trunks which accompany the arteries.

The lymph vessels begin in the mucosa just beneath the surface epithelium and pass outward through the muscularis mucosæ, into the submucosa, where they form a plexus at its outer edge. Large vessels are given off from this plexus, which pass through the muscular coats and join the large lymph vessels which run with the arteries and veins.

Lymphatic tissue, in the form of the *solitary lymph nodules,* occurs in the submucosa. In a portion of the small intestine large collections of lymph nodules, Peyer's patches, are found.

The nerves consist chiefly of the non-medullated fibres. After passing through the external muscular layer the fibres form a plexus, Auerbach's plexus, the fibres of which are in connection with nerve cells. Fibres from this plexus pass through the internal muscular layer and on reaching the submucosa form Meissner's plexus. From this plexus nerve fibres are given off which have been traced into the mucosa, but not to their termination.

THE STOMACH.

The mucous membrane of the stomach is thrown up into irregular ridges, the *rugæ,* which becomes obliterated when the organ is distended. Its surface is marked with polygonal-shaped depressions, the *gastric crypts,* formed by the infolding of the surface epithelium. At the pyloric end these crypts are very deep, extending, at times, through one-half of the thickness of the mucous membrane. The epithelium lining the crypts is a continuation of the surface epithelium. It is high, narrow, cylindrical, and rests on a thin membrana propria. The cytoplasm near the free surface of the cells is clear and contains a muco-albuminous substance, while that at the base of the cells is granular and contains oval-shaped nuclei. From three to seven glands open into these crypts.

The glands of the mucous membrane are of two kinds, **pyloric** and **peptic** (fundus or cardiac).

The **pyloric glands** are confined to a narrow zone around the pylorus. They are branched, tubular glands, the deeper portions often being tortuous. They are lined with low cylindrical epithelium, the cytoplasm being moderately clear. Each cell has a spherical-shaped nucleus, which at times may become flattened, situated at its base.

The **peptic glands** are tubular in shape with a somewhat club-shaped extremity. They open into the crypts by a narrow *neck.* The main portion of the gland is known as the *body,* and the dilated blind extremity as the *fundus.* They are packed very close

to each other, being separated by very thin bands of the stroma. Two or more of the glands open into a single crypt. The lining epithelium consists of two kinds of cells, the **chief cells** and the parietal or **acid cells.**

The **chief cells** are low cylindrical or columnar in shape. The cytoplasm is moderately clear and contains a spherical-shaped nucleus. The **acid cells** are oval or triangular in shape, with granular cytoplasm which stains intensely with the anilin dyes. Each cell contains a round or oval-shaped nucleus. In the neck and body of the gland the acid cells are numerous, lying in rows beside the chief cells. At the fundus the chief cells are the more numerous, the acid cells being more scattered and are crowded to the periphery, lying between the chief cells and the membrana propria, forming protuberances. The acid cells are surrounded by a network of *secretory capillaries,* which, uniting, form a short canal that passes out between the chief cells to the lumen of the gland. These capillaries are only to be seen when impregnated by the Golgi silver method.

The muscularis mucosæ consists of two layers of smooth muscle, an outer or longitudinal and an inner or circular. At times a third oblique layer occurs. Thin strands are given off from the muscularis mucosæ, which pass out between the glands.

The stroma is composed of connective tissue with a number of leucocytes. On account of the large number of glands it is scanty in amount.

Solitary lymph nodules are sometimes found in the mucous membrane. They are situated chiefly in the pyloric end.

The submucosa is composed of loose connective tissue and may contain fat cells.

The muscular coat consists of three layers, not always sharply defined in all parts of the walls.

THE SMALL INTESTINE.

The mucous membrane of the small intestine is thrown up into circular folds, the *valvulæ conniventes,* the surfaces of which are covered with numerous projections, the **villi.** These villi are leaf-shaped in the duodenum, roundish in the jejunum, and club-

shaped in the ileum. Surrounding the attached portion of the villi are tubular glands, the *crypts of Lieberkühn,* which dip down into the stroma, reaching nearly to the muscularis mucosæ.

A **villus** is composed of a central axis of reticular tissue containing numerous lymph cells. The surface of this axis is covered by a membrana propria homogeneous in structure. Upon this rests a single layer of cylindrical epithelial cells continuous over the entire surface of the villus and dipping down into the tubular glands at its base. The cytoplasm of the epithelial cells is granular and contains an oval-shaped nucleus situated near the base of the cell. The free border of the cells has a well-marked striated cuticula. The fusing of the cuticulæ of adjoining cells forms the cuticular membrane. Mucous or goblet cells, in various stages of development, occur among the surface epithelial cells. Leucocytes are often found between the cells of the surface epithelium.

Each villus contains a terminal lymph vessel, the lacteal, which usually runs up through its centre and terminates in an elongated pouch-like dilatation. Some of the broader villi contain two lacteals, which join at the apex of the villus. The lacteals pass through the stroma at the base of the villus, pierce the muscularis mucosæ, and empty into the plexus of lymphatics on the inner surface of the circular muscular layer.

The blood vessels of the mucous membrane are composed of two sets, the arteries of the villi and the arteries of the glands. The arteries of the villi are branches given off from the large vessels of the submucosa. They pass through the muscularis mucosæ into the villi and continue on one side of the villi to the summit, giving off capillaries which pass over and join the vein on the opposite side. The arteries of the glands are branches of vessels lying nearer the muscularis mucosæ, through which they pass and then break up into capillary networks around the glands; these form veins which pass into the submucosa.

The muscularis muscosæ consists of two layers, longitudinal and circular. It gives off strands which pass into the villi.

The submucosa has the same structure as in the other portions of the gastro-intestinal canal.

Lymph nodules are found distributed throughout the mucous membrane either as single ones, the *solitary nodules,* or in col-

lections, *Peyer's patches.* The solitary nodules are usually pear-shaped and are situated deep in the mucosa and resting on the muscularis mucosæ. In some cases where they are large they perforate the muscularis mucosæ. The villi are absent over their surface and the tubular glands are displaced.

Peyer's patches are collections of lymph nodules varying in number from ten to sixty. They are round or oval in shape and are situated opposite to the attachment of the mesentery, their long axes being parallel with the axis of the intestine. They are most numerous in the ileum and jejunum. The nodules are packed closely together and lie chiefly in the submucosa. Their pointed ends break through the muscularis mucosæ and project into the mucous membrane. Like the solitary nodules, their free surfaces are not covered by the villi, but with a single layer of cylindrical epithelium.

THE LARGE INTESTINE.

The large intestine has the same coats as the small intestine. The mucous membrane is without villi. The glands are of the simple tubular type. They are packed closely together in the stroma and are lined with high cylindrical epithelial cells. The glands contain a large number of mucous or goblet cells.

Practical Study.

Large Intestine.—A piece of the large intestine of a recently-killed dog, about three to four inches long, is cut out, then laid open and the surface of the mucous membrane carefully washed with physiological salt solution (see General Technique). It is then pinned out on sheet cork, mucous membrane uppermost, and fixed in formalin-Müller's fluid, hardened in alcohol, embedded in celloidin, and sections cut perpendicular to the surface of the mucous membrane. The sections are stained **double** and mounted in **balsam.**

Low Power.—Select a place in the section where the glands are cut longitudinally. Identify the various coats and make an outline drawing of them.

High Power.—1. Observe the structure of the **serous coat.**

2. Observe the structure of the **muscular coat,** its two layers. Is the section a *longitudinal* or *transverse* one of the intestine?

3. Observe the structure of the **submucosa.**

4. Observe the structure of the **mucous membrane.** *(a)* The **muscularis mucosæ.** *(b)* The **stroma.** How does it differ in structure from the submucosa? *(c)* The **tubular glands.** Note the lining *epithelium* resting on the *membrana propria,* also the *mucous cells.*

Draw the details of a *tubular gland* and of the *stroma.*

5. Examine the section and see if it contains any of the *solitary lymph nodules.* If so, make a drawing, with the **low power,** showing its situation.

Small Intestine.—The small intestine is prepared in the same manner as the large intestine. The human intestine is to be used, if possible, but, owing to postmortem changes, it is difficult to obtain a specimen that will show details of structure properly. The intestine of a dog or cat may be used.

Sections are stained **double** and mounted in **eosin-glycerin.**

Low Power.—Observe the various coats of the intestine. Note the folds of the *mucous membrane,* the **valvulæ conniventes,** and that their surface is studded with finger-like projections, the **villi.** Note **mucous glands** or **crypts of Lieberkühn.** See if a solitary lymph nodule shows.

How does the mucous membrane differ from that of the large intestine? Make a drawing of the *mucous membrane* showing two or more *villi* in longitudinal section.

High Power.—1. Observe the *epithelium* covering the surface of the *villi.* Note its character, the presence of *mucous cells,* and that it is continuous with the epithelium lining the *crypts of Lieberkühn.*

2. Observe the structure of the *axis of the villus,* the sections of the blood vessels and of the *lacteal.*

Make a drawing showing the *minute structure of a villus.*

Peyer's Patch.—A portion of the ileum of a dog containing a Peyer's patch is removed, opened up longitudinally along the attachment of the mesentery, and then treated in the same manner as the large intestine. Transverse sections through Peyer's patch are made, stained **double,** and mounted in **balsam.**

Low Power.—Observe the collection of *lymph nodules* which forms **Peyer's patch.** Note that they are embedded in the *submucosa* and that their pointed ends project into the mucous membrane between the villi, also that these ends are covered with a single layer of *cylindrical epithelium.* Make a drawing showing all the *coats* of the intestine and the *situation of Peyer's patch.*

The Stomach.—The stomach of a dog is removed together with a portion of the duodenum. It is slit up along the lesser curvature and its contents carefully removed. Pieces through the entire wall are cut out of both the fundus and pyloric region. These are pinned out on sheet cork, mucous membrane uppermost, and fixed in Zenker's fluid. After hardening in alcohol small pieces are embedded in celloidin and sections cut perpendicular to the surface of the mucous membrane. The sections are stained **double** and mounted in **balsam.**

Section from the Pylorus. **Low Power.**—1. Observe the relations, relative thickness, and structure of the various coats. Select a place in the section where the *glands* are cut *longitudinally.* Observe the *gastric crypts;* the various shaped sections of the *tubular glands.* Note the situation of the *stroma.*

2. See if the section contains a *lymph nodule.*

Make an outline drawing of the mucous membrane showing two or more of the glands.

High Power.—Observe the epithelium lining the crypts, and note that it is continuous with that on the surface of the mucous membrane; the epithelium of the glands. Note the transverse sections of the epithelial cells in some of the crypts.

Draw the details of a *gland.*

Section from the Fundus. **Low Power.**—Observe the *mucous membrane.* Note the **peptic glands** packed closely together with an exceedingly small amount of stroma between them, and their division into *neck, body,* and *fundus.* Also note that two or more glands open into the gastric crypt.

Make a drawing of a number of glands which are cut *longitudinally.*

High Power.—1. Observe the *epithelium* in the crypt.

2. The *epithelium* of the *neck, body,* and *fundus* of the gland.

Note the position of the **acid cells,** stained deep red, and of the **chief cells,** clear in appearance.

Make a drawing showing the details of structure of the *crypt* and of the *body* and *fundus* of a gland.

How do these glands differ in structure from the pyloric glands?

3. Select a *transverse section* of a peptic gland and make a **high-power** drawing of it.

The Œsophagus.—Pieces of the human œsophagus or that of a dog are prepared in the same manner as the large intestine. Sections are stained **double** and mounted in **balsam.**

Low Power.—Observe the relation and relative thickness of the various coats and make an outline drawing of them.

High Power.—Observe, 1, the structure of the *mucous membrane.* Note the *stratified epithelium* on its surface; the papillæ of the stroma; the *muscularis mucosæ.*

2, The *submucosa,* which may contain mucous glands.

3, The *muscular coat,* its two layers. Note that it may be composed of a mixture of *smooth* and *striated* muscle.

4, The *fibrous coat.*

Draw the details of each coat.

The Mucous Membrane of the Tongue.—The human tongue is fixed in formalin-Müller's fluid and hardened in alcohol. Blocks are cut from the posterior surface, including the *circumvallate papillæ,* and from the tip of the tongue which contains the *fungiform* and *filiform papillæ.* These blocks are impregnated in celloidin and embedded in a paper box in such a manner that sections of the three forms of papillæ may be cut at the same time. Longitudinal sections of the papillæ are cut, stained **double,** and mounted in **balsam.**

Low Power.—Observe the shape of the **circumvallate papillæ,** the **filiform papillæ,** the **fungiform papillæ.** Make an outline drawing of each form.

High Power.—Observe, 1, the structure of the *circumvallate papilla.* Note the *secondary* papillæ; the *epithelium* covering the surface; and the *taste-buds* embedded in the epithelium on its sides. Also note the *glands* in the muscular tissue, that they are of two kinds, *mucous* and *serous.*

2, The structure of a *filiform papilla.*

3, The structure of a *fungiform papilla.*

Make a drawing of each form of papilla showing its minute structure.

THE SALIVARY GLANDS.

The salivary glands are the *parotid, submaxillary,* and *sublingual.* They are all of the compound tubular type. They produce either a mucous or serous secretion, and in some cases one gland will produce both. The glands are grouped in accordance with the secretion into *mucous salivary glands* (sublingual in man, rabbit, dog, and cat); *serous salivary glands* (parotid in man, rabbit, dog, and cat); *mixed salivary glands* (submaxillary in man, ape, and mouse). The submaxillary gland in man being of the mixed kind, we shall study it alone.

The **submaxillary gland,** being of the compound type, is composed of *lobes* and *lobules.* The entire gland is surrounded by a connective-tissue *capsule.* Septa given off from this pass into the gland and surround the lobes and lobules. The lobules contain two kinds of **alveoli** or **acini,** mucous and serous.

The gland has an *excretory duct,* Wharton's duct. It divides into branches which pass between the lobes, the *interlobar ducts;* these give off branches which pass between the lobules, the *interlobular ducts;* branches from these pass into the lobules, the *intralobular ducts,* and terminate in short, narrow tubules, the *terminal ducts* (intermediary ducts or tubules), around the ends of which are grouped the *acini* or *alveoli.* The ducts are lined with epithelium resting on a homogeneous membrana propria. The character of the epithelium varies in the different divisions of the ducts. The mouth of the excretory duct is lined with stratified cylindrical epithelium consisting of two layers of cells. The remainder of the excretory duct, the interlobar and interlobular ducts, are lined with cylindrical epithelium having two rows of nuclei (pseudo-stratified). The intralobular ducts are lined with a single layer of low cylindrical epithelium, the basal portion of the cells being *striated* or *rodded.* The epithelium in the terminal ducts may be either low cuboidal or flattened.

The **serous acini** are lined with low cylindrical or cuboidal epi-

thelium. The appearance of the cytoplasm differs with the activity of the cell. The resting cells have a slight granular cytoplasm, the granules being separated by a considerable quantity of a clear substance (paraplasm); the nuclei are irregular in shape. When the cells commence to become active the cytoplasm becomes very granular and dark in appearance; the nuclei become spherical in shape. Lying between the membrana propria and the glandular cells are the *basket cells,* branched cells which anastomose with each other. They are the supporting cells for the secretory cells.

The **mucous acini** are fewer in number and more irregular in shape than the serous. They contain two kinds of cells. *(a)* Mucous cells, which in the active state are large, with a clear cell body, and have an oval or flattened nucleus situated near the base of the cell. In the resting state these cells are smaller, the cytoplasm cloudy, and the nucleus is situated nearer the centre of the cell. *(b)* Cells which resemble the serous cells in structure. They are grouped in crescentic-shaped masses between the membrana propria and the mucous cells. They stain deeply with eosin and are known as the *crescents of Gianuzzi* or the *demilunes of Heidenhain.*

Practical Study.

Human Submaxillary Gland.—Small pieces of the gland, taken as soon after death as possible, are fixed in Zenker's fluid, hardened in alcohol, and embedded in celloidin. Sections are stained **double** and mounted in **balsam.**

Low Power.—A. Observe the **lobules** separated from each other by fine **septa** of connective tissue.

B. Note that the lobules are made up of two kinds of **acini** or **alveoli,** the **mucous** and **serous.**

Make an outline drawing of a lobule and its acini.

High Power.—A. Select a **mucous acinus.** Note its irregular shape; its lining of *mucous cells,* their shape, the character of the cell body; the shape and position of the nucleus; the **crescents of Gianuzzi** or **demilunes of Heidenhain,** a mass of deeply stained cells, with spherical nuclei, lying outside of the mucous cells and next to the membrana propria.

Make a drawing showing all the details of structure.

B. Select a *transverse section* of a **serous acinus.** Note its shape; its lining of epithelial cells, with granular cell body; the shape and position of the nucleus.

Make a drawing showing all the details of structure.

C. Select an **intralobular duct.** Note its size, its membrana propria, its lining epithelium.

Make a drawing showing all the details of structure.

D. Select an **interlobular duct** and proceed as under **C.**

THE PANCREAS.

The pancreas is built up on the plan of a compound tubular gland. It is composed of a large number of small *lobules* surrounded by connective tissue. The excretory duct, the duct of Wirsung, runs the entire length of the gland and is embedded in its substance. After leaving the pancreas it joins the bile duct, and they empty by a common opening on the internal surface of the duodenum.

The **acini** are grouped around an intermediate tubule which empties into a short intralobular duct. The epithelium of the acini is low cylindrical or conical in shape. The cytoplasm is divided into two distinct zones, the internal or granular, and the peripheral or homogeneous. The width of these zones varies according as the cell is active or resting. During the active state the granular zone becomes smaller and may disappear entirely, while the homogeneous becomes larger; in the resting state the zones are of about equal size. The cell decreases in volume in the active state and returns to its original size during the period of rest. The acini also contain polygonal or stellate cells, the *centro-acinal cells,* or the *cells of Langerhans.* At present there are a number of opinions as to the significance of these cells.

Lying between the acini are groups of slightly granular cells arranged in anastomosing trabeculæ surrounding irregular spaces which contain a large number of capillaries. These are the *areas of Langerhans.* It is supposed that these cells produce a secretion which is taken up by the capillaries.

The excretory duct is lined with a single layer of cylindrical epithelium resting on a membrana propria which becomes shorter in the smaller ducts and finally flat in the terminal ones.

The blood supply is rich.

Practical Study.

The human pancreas, taken as soon after death as possible, or that of a recently-killed dog, is cut into thin transverse slices and fixed in Zenker's fluid. After hardening in alcohol, embed in celloidin. Cut transverse sections through the organ, stain them **double,** and mount them in **balsam.**

Low Power.—Observe the capsule of connective tissue and the septa between the lobules; the collections of acini; and the intralobular duct. Make a drawing showing the shape of the acini.

High Power.—Select an acinus; observe the lining epithelial cells and their minute structure. Is the acinus active or in a state of rest? Note the *centro-acinal cells* and the *areas of Langerhans;* also the intralobular ducts and the intermediate tubules.

Make a drawing showing all details of structure.

THE LIVER.

The liver is a compound tubular gland with anastomosing tubules. It is composed of *lobes* and numerous *lobules.* The latter are polyhedral in shape and average 1 mm. in diameter. The organ is surrounded by a connective-tissue *capsule,* which is continued into the interior at the portal fissure and surrounds the *portal vein, hepatic artery,* and *bile duct,* forming the *capsule of Glisson.* In the pig the capsule of Glisson completely surrounds the lobules; in man, only partially.

The portal vein and hepatic artery enter and the bile duct leave the liver at the portal fissure. Within the organ they are surrounded by Glisson's capsule, forming the so-called *portal canal.*

The **portal vein** divides and subdivides, its terminal branches passing between the lobules, forming the *interlobular veins.* The hepatic artery and bile duct follow the course of the portal vein.

The interlobular veins give off branches which enter the lobules,

where they break up into a long, narrow-meshed capillary network, between which are rows or *cords of liver cells.*

In the long axis of the lobule is the *central* or *intralobular vein.* This is formed by converging capillaries from the fundus of the lobule, and it passes down to its base, where it enters the *sublobular vein,* one of the branches of the *hepatic vein,* which carries the blood out of the liver. During its course through the lobule the central vein gives off capillaries which become continuous with those of the interlobular veins.

The **hepatic artery** breaks up into capillaries which are distributed to the connective tissue and the walls of the other vessels. These capillaries pass into those given off from the interlobular veins.

The **hepatic veins** are formed by the joining of the sublobular veins. They are surrounded by a thin layer of connective tissue, and are always found running by themselves, not being accompanied by the other vessels.

The **bile ducts** are formed between the lobules by the union of the bile capillaries. The ducts follow the course of the portal vein, gradually growing larger, and emerge from the portal fissure as the hepatic ducts.

The **liver cells** are of an irregular polyhedral shape, with a granular cytoplasm which may contain pigment and fat granules. Their nuclei are spherical in shape, and a cell may have two nuclei. The cells are arranged in rows, forming the *hepatic cords,* which anastomose with each other and occupy the spaces between the blood capillaries. The cells enclose a minute tubular channel, the *bile capillary.* The angles of the cells are grooved for the reception of the blood capillaries.

The **bile capillaries** are minute channels between the hepatic cells. They have no distinct walls. They correspond to the lumen of a tubular gland, and in the human liver they lie usually between two rows of liver cells, being separated from the blood capillaries by at least one-half the diameter of a liver cell. They form networks which correspond in size and shape to that of the liver cells. They follow the course of the hepatic cords and at the periphery of the lobule pass into the small bile ducts.

The **bile ducts** have a membrana propria and are lined inter-

nally with a single layer of epithelial cells. In the smaller ducts this epithelium is flat, becoming cuboidal and cylindrical as the ducts increase in size. The larger ducts have, in addition to the membrana propria, an external connective-tissue layer. Smooth muscle occurs in the walls of the largest ducts.

The connective-tissue elements within the lobule consist of a large number of exceedingly fine fibres (*Gitterfasern*), which surround the blood capillaries. They are not seen in specimens prepared by the ordinary methods, but require special methods for their demonstration.

The lymph vessels accompany the portal and hepatic veins. They pass into the lobules and form perivascular lymph spaces around the blood capillaries.

Practical Study.

Pig's Liver.—Small pieces of the liver of the pig are fixed in formalin-Müller's fluid, hardened in alcohol, and embedded in celloidin. Sections are stained **double** and mounted in **balsam.**

Low Power.—Observe the *lobules* surrounded by the septa of connective tissue, prolongations of *Glisson's capsule.*

Note the **central vein** (intralobular vein); the radiating capillaries; the cords of liver cells. Also note that at the junction of two or more lobules the connective tissue becomes somewhat rectangular in shape and that it has embedded in it sections of the **portal vein, hepatic artery,** and **bile duct.**

Make a drawing of two or more lobules showing the above-described parts.

Human Liver.—Small pieces of the human liver are treated in the same manner as the pig's liver. Sections are stained **double** and mounted in **balsam.**

Low Power.—Observe the general structure. Note, 1, that the **lobules** are not sharply marked out by the connective-tissue septa as in the pig's liver, but gradually merge into each other; 2, the *central vein* from which the capillaries radiate, and the cords of liver cells between the capillaries; 3, the sections of the *portal vein, hepatic artery,* and *bile duct* embedded in a small mass of connective tissue at the periphery of the lobules.

Make a drawing of a single lobule.

High Power.—Observe, 1, the shape, structure, and arrangement of the liver cells; 2, the grouping and structure of the sections of the *portal vein, hepatic artery,* and *bile duct.*

Make a drawing of at least two cords of liver cells showing their minute structure and their relations to the capillaries. Also a drawing showing the minute structure of the portal vein, hepatic artery, and bile duct.

Bile Capillaries.—For demonstrating the bile capillaries the Golgi chrome-silver method of impregnation may be employed. This method does not give a uniform impregnation throughout, but usually a few lobules just beneath the surfaces of the blocks of tissue will show the bile capillaries of a few lobules impregnated.

Fresh pieces of liver tissue are placed for three days in the following mixture: potassium dichromate, 4 per cent. aqueous solution, 4 volumes; osmic acid, 1 per cent. aqueous solution, 1 volume. They are then placed directly into a 0.75 per cent. aqueous solution of silver nitrate, which is renewed at the end of an hour. and allowed to remain for from two to three days. They are then washed in water, dehydrated in strong alcohol, and embedded in celloidin. Sections are made, cleared in xylol, and mounted in balsam.

Low Power.—Observe the network of the *bile capillaries,* which show as fine black lines. Note that they radiate from the centre of the lobule and follow the course of the hepatic cords.

High Power.—Note that the network of capillaries outlines the liver cells, the latter being unstained.

Make a drawing showing the appearance of the network of capillaries.

Specimens prepared as above will keep for a while, but they gradually deteriorate.

THE KIDNEY.

The kidney is a compound tubular gland made up of numerous branched tubules, the *uriniferous tubules.* It is surrounded by a thin *capsule* of connective tissue, the deeper portion of which contains a thin layer of smooth muscle. In some animals the kidney consists of a single *renculus,* while the human kidney is made up of a number of *renculi.* In the fœtus these renculi are distinct, being separated by bands of connective tissue, but in adult life they merge into one another and no sharp line of division between is visible. In a few instances the fœtal condition persists in adult life, forming the lobulated kidney.

In a longitudinal section of a kidney two distinct zones can be made out—an outer or *cortical* and an inner or *medullary zone.*

The **cortical zone** is subdivided into the *cortical pyramids* (labyrinth) and *medullary rays* (pyramids of Ferrein). The cortical pyramids contain convoluted tubules and *Malpighian bodies.* The medullary rays are made up of *parallel tubules* running a straight or slightly wavy course.

The **medulla,** or **Malpighian pyramid,** is of a pyramidal shape, with its base directed toward the cortex, and from which is given off the medullary rays, its apex, or papilla, projecting into the pelvis of the kidney.

The **uriniferous tubules** are the secreting portion of the kidney. They have a thin, homogeneous membrana propria, and are lined with a single layer of epithelial cells, the shape and structure of which vary in the different divisions of the tubules.

A uriniferous tubule is divided into the following divisions: (1) the *Malpighian body;* (2) the *first* or *proximal convoluted tubule;* (3) the *descending arm of Henle's loop;* (4) the *loop of*

Henle; (5) the *ascending arm of Henle's loop;* (6) the *second, distal,* or *intercalated convoluted tubule;* (7) the *arched tubule;* (8) the *straight* or *collecting tubule;* (9) the *duct of Bellini.*

The **Malpighian body** is situated in a cortical pyramid. It is spherical in shape, having a diameter of from 120 μ to 200 μ. It consists of two parts: a lobulated mass of blood vessels, the *glomerulus;* and the dilated and invaginated end of a uriniferous tubule, the *capsule of Bowman,* which completely surrounds the glomerulus. A narrow space exists between the inner wall which covers the surface of the glomerulus and the outer wall (capsule of Bowman). This space is continuous with the lumen of the first convoluted tubule. At one side of the Malpighian body an artery—*afferent artery*—passes through the capsule and breaks up into tufts of capillary blood vessels—the *glomerulus;* these capillaries, uniting, form the *efferent vessel,* which passes out alongside of the afferent artery. The capsule of Bowman is lined with a single layer of flat epithelial cells with projecting nuclei. The surface of the glomerulus is covered with a similar layer of epithelium. The epithelium of the capsule is continuous with that lining the neck of the convoluted tubule, which is cuboidal in shape.

The **first** or **proximal convoluted tubule** is situated in a *cortical pyramid.* It measures from 40 μ to 70 μ in diameter and is lined with a single layer of low cylindrical epithelium. The cytoplasm of this epithelium is striated, the striation being more marked at the base of the cell. The line of demarcation between the cells is not distinct. The free surface of the cells, in well-preserved preparations, has a "bush-like" appearance resembling somewhat short, thick cilia.

The **descending arm of Henle's loop** is situated in the base of the *medullary ray* and in the *medulla.* It is from 9 μ to 15 μ in diameter. The lining epithelium is flat, the cell body bulging at the situation of the nucleus, giving the lumen a wavy appearance. The cytoplasm of the cells is moderately clear.

The **loop of Henle** is situated in deeper portions of the *medulla.* The lining epithelium may be flat, like that in the descending arm, or cuboidal, like that of the ascending arm.

The **ascending arm** is situated in the *medulla* and *medullary ray.* It measures from 23 μ to 28 μ in diameter, and is lined with

cuboidal-shaped epithelium with striated cytoplasm similar to that of the first convoluted tubule.

The **distal** or **intercalated tubule** is situated in a *cortical pyramid*. It is from 39 μ to 45 μ in diameter; shorter than the first convoluted tubule, having but three to four convolutions. The lining epithelium is similar in structure to that of the first convoluted tubule, the height of the cells and the size of the nuclei being greater.

The **arched tubule** is situated partially in a *cortical pyramid* and partially in a *medullary ray*. It is lined with cuboidal epithelium, the cytoplasm of which is clear.

The **collecting tubules** start at the *apex of a medullary ray*, pass into the *medulla*, then into the *papilla*, where they are known as the *ducts of Bellini*, and open on its surface. At their commencement they average 45 μ in diameter; as they grow larger their diameter increases to about 75 , and the ducts of Bellini have a diameter of from 200 μ to 300 μ. The epithelium lining them is cuboidal in shape in the smaller tubules, which gradually increase in height, until in the ducts of Bellini it becomes high cylindrical. The cytoplasm of the cells is clear. The lumen of these tubules is wide.

RECAPITULATION.

Portion of Tubule.	*Character of Epithelium.*	*Position of Tubule.*
Capsule of Malpighian body.	Flat, with bulging nuclei.	Cortical pyramid.
Proximal convoluted.	Striated cuboidal.	Cortical pyramid.
Descending arm	Clear, flat.	Medulla.
Henle's loop.	Clear, flat, or striated cuboidal.	Deep portion of medulla.
Ascending arm.	Striated cuboidal.	Medulla and medullary ray.
Distal convoluted.	Same as proximal; cells larger.	Cortical pyramid.
Arched	Clear, cuboidal.	Cortical pyramid and medullary ray.
Collecting.	Clear, cuboidal, or cylindrical.	Medullary ray and medulla.
Duct of Bellini	Clear, high cylindrical.	Papilla.

Blood Supply.—The renal artery enters, and the renal vein leaves, the kidney at the hilus. The artery breaks up into branches,

the columns of *Bertini*, which pass up between the lobules (renculi) of the kidney, forming arches at the junction of the cortex and medulla. The arterial arches give off branches which pass up through the central portion of the cortical pyramids, the *interlobular arteries;* these give off lateral branches which enter the Malpighian bodies, forming the afferent artery. The efferent vessel of the Malpighian body breaks up into capillaries which surround the tubules of the cortex. These capillaries, uniting, form small veins which empty into the interlobular vein. The *interlobular vein* is formed from a stellate group of veins—*stellulæ Verheyanii*—which lie beneath the capsule. The vein passes down through the cortical pyramid and empties into the venous arch.

From the under surface of the arterial arch branches are given off which pass down into the medulla, forming the *vasa recta,* or straight vessels. These form a long-meshed capillary network around the tubules. Another set of vessels, the *false vasa recta,* are given off from Malpighian bodies nearest to the medulla. The capillaries from these two sets of vessels, uniting, form veins which pass up and empty into the venous arches.

The connective tissue found in the kidney is slight in amount, the largest masses being found around the larger blood vessels. The amount between the tubules, except in the papilla, is so slight that it can hardly be demonstrated.

The nerve fibres of the kidney are distributed chiefly to the blood vessels. Nerve fibres have been traced to the membrana propria of the tubules.

THE PELVIS OF THE KIDNEY AND URETER.

The wall of the pelvis of the kidney and ureter is composed of three coats—the outer fibrous, the middle muscular, and the lining mucous membrane.

The *fibrous coat* is composed of rather coarse connective-tissue fibres arranged in loosely connected bundles.

The *muscular coat* consists of two layers of smooth muscle, an

outer circular and an *inner longitudinal.* The lower portion of the ureter has a third muscular layer, an outer longitudinal.

The *mucous membrane* has a stroma composed of fine connective-tissue fibres with a large number of cells. The free surface of the mucous membrane is covered with transitional epithelium (see page 50). The submucosa is comparatively thin and merges into the stroma.

Practical Study.

Topography of the Kidney.—For this purpose the kidney of a small animal, rabbit or guinea-pig, is taken, as it is a single kidney or renculus. The kidney is fixed in formalin-Müller's fluid. After hardening in alcohol, embed in celloidin and make transverse sections through the entire organ, so as to include the papilla and pelvis. The sections are stained **double** and mounted in **balsam.**

Naked Eye.—Observe the general shape of the section and identify the *cortex, medulla, papilla,* and *pelvis.* Note that the *cortex* completely surrounds the medulla and comes down on a level with the apex of the papilla.

Make an outline drawing of the section showing the zones.

Low Power.—1. Identify the *capsule* and trace it around until it becomes lost in the wall of the *pelvis.*

2. Observe the *cortical zone* and its division into *cortical pyramids* (labyrinth) and *medullary rays* (pyramids of Ferrein).

3. Observe the *Malpighian bodies* and the *convoluted tubules* situated in the *cortical pyramids.*

4. Observe that the *medullary rays* are composed of *parallel tubules,* the *collecting tubules* and the *ascending arms of Henle's loops.* Note that these tubules pass down into the medulla.

5. Note the structure of the *medulla.* Observe that it is composed of parallel tubules, which become few in number in the *papilla,* and that they open on the surface of the papilla.

6. Observe the wall of the *pelvis* of the kidney.

Human Kidney.—Small pieces of a human kidney, removed as soon after death as possible, are fixed in Zenker's fluid for forty-

eight hours and then hardened in alcohol. Embed in celloidin and cut sections through the cortex and medulla, stain them **double,** and mount in **balsam.**

Low Power.—Identify the *capsule, cortical pyramids, medullary rays,* and *medulla.*

High Power.—1. Observe the structure of a *Malpighian body,* selecting one, if possible, showing the *afferent* and *efferent artery,* the tufts of blood vessels forming the glomerulus, and the *constricted neck* of the convoluted tubule. Note the *capsule of Bowman* lined with flat epithelial cells, and that these cells are continuous over the surface of the tufts of blood vessels.

Make a drawing showing the minute structure of a Malpighian body.

2. Observe the structure of the *convoluted portion* of the tubule. Note its membrana propria and the lining epithelium.

Make a drawing of a transverse section of a convoluted tubule showing its minute structure.

3. Observe the structure of a *medullary ray.* Note the two kinds of tubules, the *collecting* and the *ascending arms* of *Henle's loops.*

Make a drawing showing the minute structure of both tubules.

4. Pass down into the *medulla.* Note the three kinds of tubules, the *descending* and *ascending arms of Henle's loop,* the *collecting tubules.* Search through the medulla, where the tubules are cut longitudinally, and see if a *loop of Henle* can be found.

Make a drawing showing the minute structure of the above-described parts.

5. Pass down into the *papilla.* Note the large size of the *collecting tubules,* the *ducts of Bellini,* and the increase of the supporting connective tissue. The tubules of the papilla may show in transverse section.

Make a drawing of the ducts of Bellini.

6. Observe the *blood vessels.* Note the *interlobular artery* and *vein;* the *renal arches;* the *vasa recta.*

Make outline drawings of the various portions of the kidney and show the position and relation of the blood vessels to the various parts.

THE SUPRARENAL BODY.

The suprarenal body is surrounded by a capsule of connective tissue which contains a small amount of smooth muscle. Its interior is divided into a *cortical* and *medullary zone.*

The **cortical zone,** on account of the arrangement of its cellular elements, is divided into three layers—the *zona glomerulosa,* the *zona fasciculata,* and the *zona reticularis.* The zona glomerulosa is formed of oval-shaped masses of polygonal cells with a granular cytoplasm; the cells of the zona fasciculata are arranged in long, cylindrical groups; those of the zona reticularis as irregular, anastomosing cords. The cells of this layer are deeply pigmented. All of the groups of cells are surrounded by delicate connective-tissue septa derived from the capsule.

The **medullary zone** consists of polygonal-shaped cells with a finely granular cytoplasm, arranged in roundish masses and cords. Numerous nerve cells are distributed through the medulla. The medulla also contains a large number of non-medullated nerve fibres.

The arteries of the capsule divide into branches which form a long-meshed network in the cortex and then pass into the medulla, forming a round-meshed network. The capillaries of the medulla unite, forming veins of large size which pass out of the organ at the hilus. The larger veins are surrounded by bundles of smooth muscle.

Practical Study.

The suprarenal body from man or dog is fixed in formalin-Müller's fluid, hardened in alcohol, and embedded in celloidin. Sections are made through the entire organ. These are stained **double** and mounted in **balsam.**

Low Power.—Observe the arrangement of the cellular elements of the cortex and of the medulla.

Make an outline drawing showing the appearance of the cortex and medulla.

THE ORGANS OF RESPIRATION.

THE TRACHEA.

The walls of the trachea are composed of three layers—the *external* or *fibrous,* the *submucosa,* and the *mucosa.*

The **fibrous layer** is composed of coarse connective-tissue fibres and has embedded in it *incomplete rings of hyalin cartilage.* These rings are from sixteen to twenty in number and are C-shaped, the gap in their posterior portion being bridged over by a band of smooth muscle tissue. Each ring is surrounded by a *perichondrium.*

The **submucosa** consists of loose connective tissue and merges into both the fibrous layer and stroma of the mucous membrane. It contains *mucous glands* of the compound tubular type. The alveoli are lined with cylindrical epithelium. The duct of the glands opens on the surface of the mucous membrane. The most of the alveoli lie between the cartilage rings.

The **mucosa** has a stroma composed of fine, fibrillated fibres with a network of fine elastic fibres, and contains numerous leucocytes. The *basal membrane* is well developed and is homogeneous in structure. The surface of the mucosa is covered with stratified, cylindrical, ciliated epithelium. Many of the surface cells are of the mucous type.

Practical Study.

Trachea.—A piece of human trachea is cut up along its posterior surface and then pinned out on sheet cork, mucous membrane uppermost. It is then fixed in formalin-Müller's fluid, hardened in alcohol, and embedded in celloidin. Sections are made parallel to the long axis of the trachea, so as to include at least two

cartilage rings. The sections are stained **double** and mounted in **eosin-glycerin.**

Low Power.—Observe the transverse sections of the *hyalin cartilage rings* embedded in the *fibrous layer;* the *submucosa;* the *mucosa* with its *epithelium.*

Make an outline drawing showing all the layers.

High Power.—Observe: 1. The *mucous membrane,* its stratified, cylindrical, ciliated epithelium resting on the basal membrane. Note the presence of the mucous cells.

2. The structure of the *stroma* of the mucous membrane.

3. The structure of the *submucosa.*

4. The structure of the mucous glands. See if a duct shows.

5. The structure of the *fibrous layer* and the *hyalin cartilage rings.*

Make a drawing of a section through the wall of the trachea showing the minute structure of all its layers; also of a mucous gland.

THE BRONCHI AND LUNGS.

The trachea at its lower end divides into two branches, the *main bronchi.* These have the same structure as the trachea.

The main bronchi divide into branches, the *large bronchi,* which enter the lungs and divide and subdivide, growing smaller and smaller, and finally end in the terminal bronchi. As the bronchi decrease in size, various elements entering into the formation of their walls gradually disappear. This permits of the classification of the bronchi, as regards the structure of their walls, into *large bronchi, medium bronchi,* and *terminal bronchi.*

The walls of a **large bronchus** are composed of an external fibrous layer, in which are embedded irregular *plates* of hyalin cartilage; a muscular layer, composed of smooth muscle, which forms a complete ring; a submucosa of connective tissue; and a mucous membrane, the surface of which is covered by stratified, cylindrical, ciliated epithelium. Mucous glands are found in the submucosa. The large bronchi are surrounded by a mass of loose connective tissue which contains branches of the *pulmonary artery, pulmonary vein, bronchial artery,* and *bronchial nerve.*

The walls of a **medium bronchus** consist of a fibrous layer *without* cartilage plates; a submucosa which is much thinner and is without mucous glands; a muscular layer which is complete but thin; and a mucous membrane the surface of which is covered with a single layer of cylindrical, ciliated epithelium. The pulmonary blood vessels are usually at some distance from the bronchus.

A **terminal bronchus** (respiratory bronchus) has thin walls which consist of a fibrous layer containing a few scattered smooth muscle cells, and it is lined with a single layer of mixed epithelium, which in the commencement of the bronchus may be low ciliated cylindrical. This soon changes into cuboidal, which is grouped in irregular patches between which are thin, non-nucleated plates of *respiratory epithelium.*

The **lungs** are divided into *lobes,* these being made up of *lobules.* The surface of the lungs is covered with a serous membrane, the *visceral pleura,* which at the root of the lungs is reflected on the inner surface of the thoracic walls, forming the *parietal pleura.*

The **lobules** are of an irregular polygonal shape and are separated from each other by a thin layer of connective tissue, the *interlobular connective tissue.* In the adult this tissue contains considerable black pigment, inhaled carbon. The small bronchi enter the lobules, generally at the apex, and break up into branches, the terminal bronchi. From these are given off, in an irregular manner, tubular prolongations, the *air passages* (infundibula, alveolar passages), which branch and anastomose. From the air passages are given off the *air vesicles* or *alveoli.*

The walls of the air passages are composed of fine fibrillated and elastic fibres with a few smooth muscle cells. The lining epithelium is chiefly of the respiratory type, with a number of cuboidal cells (fœtal cells) with a granular cytoplasm. The proportion of the two kinds of cells varies.

The **air vesicles** are lined with the two kinds of epithelium as in the air passages. The wall is composed of fibrillated and elastic fibres almost entirely, though a few smooth muscle cells may be present. The elastic fibres are arranged in a circular manner around the opening of the air vesicle into the air passage. The

rings of elastic fibres of adjoining air vesicles, fusing, form the *alveolar septa.*

The **blood vessels** of the lungs follow the general course of the bronchi. The pulmonary artery, after entering the lungs, breaks up into branches which follow the branches of the bronchi. A small branch enters the lobules with the bronchus and then divides into branches, one of which goes to each air passage; small branches from these go to the air vesicles and break up into a dense capillary network in their walls. The air passages are supplied with a similar capillary network. This capillary network lies close to the inner surface of the air vesicle and in direct contact with the respiratory epithelium. The veins which received the blood from the capillaries of the air vesicles run in the interlobular connective tissue and, uniting, form the larger pulmonary veins.

The bronchial arteries supply the walls of the bronchi. They break up into capillary networks which surround the bronchi. These capillaries anastomose with those of the pulmonary vessels, or unite to form small veins, the bronchial veins, which empty into the pulmonary veins.

The **lymphatics** consist of two sets of vessels which originate in the alveolar septa. One set passes into the interlobular connective tissue and becomes continuous with those of the pleura, which terminate in the lymph nodes at the root of the lung. The other set accompany the pulmonary artery and also terminate in the lymph nodes at the root of the lung.

PRACTICAL STUDY.

Dog's Lung.—The lungs of a dog, together with the trachea, are carefully removed and a canula is tied in the trachea. The canula is connected with a funnel by a rubber tube and the lungs distended by pouring formalin-Müller's fluid into the funnel. When the lungs have become fully distended, a ligature is placed around the trachea and the lungs immersed in the same fluid for forty-eight hours. They are then cut in pieces, washed well in running water, and hardened in alcohol. After embedding in celloidin, sections are made so as to include a transverse section of a large bronchus. The sections are stained **double** and mounted in **balsam.**

Low Power.—1. Select a large bronchus and note its relations to the pulmonary blood vessels. Note its fibrous coat, in which are embedded sections of plates of hyalin cartilage; the submucosa; the muscular layer; the mucous membrane; the mucous glands.

Make an outline drawing showing all the layers of the bronchus and its relation to the pulmonary blood vessels.

High Power.—Observe the structure of the mucous membrane, the muscular coat, the submucosa, the mucous glands, and the fibrous coat. Note the bronchial artery and nerve.

Make a drawing of a section through the wall of the bronchus, showing the minute structure of its coats.

2. Select a *medium bronchus* and proceed as under 1.

3. **Low Power.**—Select a *terminal bronchus,* either in transverse or longitudinal section, from which an air passage is given off. Note the irregular shape of the air passage, its course, and the openings of the air vesicles into it.

Make an outline drawing of the bronchus, air passage, and air vesicles.

High Power.—Note the structure of the wall of the bronchus and air passage.

Make a drawing of the wall of the bronchus showing its minute structure.

Human Lung.—A human lung is prepared in the same manner as the dog's lung (see above). Sections are stained **double** and mounted in **eosin-glycerin.**

Low Power.—Observe the pleura and the interlobular connective tissue. Select an air vesicle with a "bottom" to it and turn on the

High Power.—Observe the shape of the air vesicle, its lining epithelium, the fœtal cells, the space between them which is lined with the respiratory epithelium. Note the capillary network.

Make a drawing showing the minute structure of an air vesicle.

Elastic Tissue of Air Vesicle.—Sections of human lung are stained with *Weigert's Elastic Tissue Stain* and mounted in **balsam.**

High Power.—Select an air vesicle with a "bottom" to it and note the elastic fibres (stained bluish).

Make a drawing of the elastic fibres.

Human Lung Blood Vessels Injected.—A human lung is injected through the pulmonary artery with blue gelatin. After hardening in alcohol and embedding in celloidin, rather thick sections are made. The sections are stained with **eosin** and mounted in **balsam.**

Low Power.—Select an air vesicle in which the capillaries are completely injected, and turn on the

High Power.—Note the size and arrangement of the capillaries and make a drawing of them.

THE THYROID GLAND.

The thyroid gland consists of numerous acini of a spherical or irregular shape. They are separated from each other by connective tissue. They have no excretory ducts. The acini have a thin membrana propria and are lined by a single layer of cuboidal epithelium. These cells are of two varieties, the *chief cells* and the *colloid cells.* The chief cells become converted into colloid cells, which produce the secretion of the gland, the *colloid substance.* During the secretion of the colloid substance the colloid cells become lower, and at times the cells become converted into colloid, the entire acinus becoming filled with it.

Practical Study.

Pieces of the thyroid gland of a young person are fixed in formalin-Müller's fluid, hardened in alcohol, and embedded in celloidin. Sections are stained **double** and mounted in **balsam.**

Low Power.—Observe the size, shape, and relations of the various acini; the connective tissue in which they are embedded; and the acini containing the colloid material (stained bright red).

High Power.—Select an acinus cut transversely. Note the membrana propria and the lining epithelium.

Make a drawing showing the minute structure of an acinus.

PART THIRD.

The Reproductive Organs and Sense Organs.

vasa efferentia. These become convoluted and form the **coni vasculosi,** which are packed together, forming the **head of the epididymis.** The convoluted tubules of the head gradually unite and form the **body of the epididymis;** and finally they become one convoluted tubule, forming **the tail of the epididymis;** this tubule passes over and unites with the **excretory** duct, the **vas deferens.**

Tunica Albuginea.—The tunica albuginea is divided into two layers, an **external** composed of dense fibrous tissue, and an **internal,** or **tunica vasculosa,** composed of loose connective tissue containing numerous blood vessels.

Tunica Vaginalis.—This is a serous membrane and covers the external surface of the tunica albuginea.

Convoluted Seminiferous Tubule.—Begins as a closed canal, being packed in one of the compartments formed by the trabeculæ. The convolutions are bound to each other by loose connective tissue in which there are small nerves, blood vessels, etc. In addition to these, there are irregular groups of large nucleated cells. the **interstitial cells.** Nothing is known with regard to the significance of these cells.

The tubule from without inward consists of a thin membrana propria, then a row of peripheral cells consisting of the **sustentacular cells** or **cells of Sertoli,** and large epithelial cells with nuclei rich in chromatin.

The **sustentacular cells** are high, and cylindrical in shape; their basal processes join and rest on the membrana propria. They give off processes from their sides which form a network for the support of the epithelial cells. Between the bases of the sustentacular cells are large, oval-shaped epithelial cells, the nuclei of which in the active tubules show the various stages of mitosis. These are the **spermatogonia cells,** from which the **semen** is formed. These cells divide and form the next layer of cells (toward the lumen), the **spermatocytes,** which in turn divide forming daughter cells, the **spermatids.** These cells form the inner layers and become the **spermatosomes,** from which the **spermatozoa** are formed, their heads being formed from the nuclei, their tails from the cytoplasm.

Tubuli Recti, or Straight Tubules.—These have a thin mem-

brana propria and are lined with a layer of simple, cuboidal-shaped epithelial cells.

Rete Testis.—The canals of the rete testis are lined with a single layer of cuboidal or flattened cells.

Vasa Efferentia.—These tubules have a membrana propria fibrous in appearance, and they are surrounded by two or more layers of smooth muscle. The lining epithelium consists of two kinds of cells: groups of simple, ciliated, cylindrical epithelium alternating with groups of non-ciliated, simple cuboidal epithelium.

Epididymis.—The tubules of the epididymis are lined with stratified, ciliated, cylindrical epithelium resting on a thin layer of connective tissue. Outside of this there are two thin layers of smooth muscle tissue, an inner circular and an outer longitudinal. The convolutions of the tubules are held together by a loose and vascular connective tissue.

Spermatic Cord.—The spermatic cord is composed of blood vessels, lymph vessels, nerves, and the **excretory duct** of the testicle, the **vas deferens**, all being embedded in rather loose connective tissue.

The **vas deferens** has three coats: an **external fibrous, middle** or **muscular**, and **internal** or **mucous membrane.**

The **muscular layer** is composed of smooth muscle divided into the external or longitudinal and internal or circular. A third layer is found at the commencement of the vas, a thin longitudinal one inside of the circular.

The **mucous membrane** is thrown up into longitudinal folds. Its stroma is rich in fine elastic fibres and its surface is covered with *stratified cylindrical epithelium.* At times this epithelium may be ciliated in the commencement of the vas.

THE SEMEN.

The semen is the secretion of the testicle and consists almost entirely of formed bodies, the **spermatozoa, floating in a thick** albuminous fluid.

The **spermatozoon** consists of three parts: **the head, the middle piece**, and the **tail** or **flagellum.**

The **head**, in man, measures from 3 to 5 μ in length and from

2 to 3 μ in width. Its shape, when seen on the flat, is oval; when seen on its edge, pyriform.

The **middle piece** is rectangular in shape, measuring 6 μ in length and 1 μ in width. It is attached to the base of the head.

The **tail** arises from the end of the middle piece. It is long (40 to 60 μ), tapering, and thread-like. When alive the tail vibrates in somewhat the same manner as a cilium and propels the head before it. By means of this movement the spermatozoa are enabled to move about in the genital organs of the female.

Practical Study.

Human Testicle.—A human testicle, obtained as soon after death as possible, is cut across, transversely, into slices about one-quarter of an inch thick and fixed in formalin-Müller's fluid, then hardened in alcohol. After embedding in celloidin, sections are made at right angles to the long axis of the testicle, so as to include the epididymis. The sections are stained **double** and mounted in **balsam.**

Naked Eye.—Identify the **epididymis,** the **tunica albuginea,** the **corpus Highmori,** the **collections of seminiferous tubules.**

Make an outline drawing of these parts showing their relations.

Low Power.—Observe the dense **tunica albuginea** and trace it into the **corpus Highmori.** Note the anastomosing canals of the **rete testis.** Note the various shaped sections of the convoluted tubules, also those of the **epididymis.**

High Power.—Select a *transverse section of a convoluted tubule;* note its membrana propria, the several layers of the lining epithelium. Identify the **sustentacular cells,** the **spermatogonia cells,** the **spermatocytes,** and the **spermatids.** Note that the lumen of the tubule is more or less filled with granular matter in which may be seen the **heads of the spermatozoa,** stained purple by the hæmatoxylin.

Also note the collections of **interstitial cells** between the tubules.

Make a drawing of a transverse section through the tubule, showing all details of structure.

Next pass to the **corpus Highmori.** Observe the canals of the

rete testis and their lining epithelium; the structure of the corpus Highmori itself.

Make a drawing of one of the canals of the **rete testis** showing all details of structure.

Finally pass to the **epididymis** and select a transverse section of one of the tubules. Observe the layers of smooth muscle surrounding the tubule, the connective-tissue stroma, and the lining epithelial cells.

Make a drawing showing all details of structure.

Transverse Section of the Human Spermatic Cord.—The spermatic cord is dissected off from the testicle used above and is fixed and hardened in the same manner. After embedding in celloidin, transverse sections are made through the cord. These are stained **double** and mounted in **balsam.**

Low Power.—Note the blood vessels and nerves embedded in the connective tissue; also the *transverse section* of the **vas deferens.** Note the various coats of the vas.

Make an outline drawing of the vas deferens showing its coats.

High Power.—Observe the structure of the **mucous membrane,** its **epithelium** and **stroma.** Note the structure of the two layers of the **muscular coat** and also of the **fibrous coat.**

Make a drawing of a section through the walls of the **vas** showing all details of structure.

Spermatozoa.—Human spermatozoa may be obtained from the seminal vesicles or from specimens of semen sometimes received in a laboratory for microscopic examination. They are to be fixed in a saturated aqueous solution of picric acid. After they have settled, which requires 24 hours, the picric acid solution is poured off and pure glycerin added for preserving them.

A drop of the glycerin is placed on a slide, covered, and examined with the

Low Power.—Find a granular-looking mass and place it in the centre of the field of the microscope and turn on the

High Power.—Select an isolated **spermatozoon** and note its three divisions, the **head, middle piece,** and **tail.**

Make a drawing of a single spermatozoon with its head as seen on the flat, and of another with its head seen on edge.

For studying the movements of the living spermatozoa an ani-

mal is killed and the testicle removed. Then with a sharp knife a small incision is made into the head of the epididymis, and a small drop of the milky fluid which exudes is mixed with a drop of sodium chlorid solution on a slide, covered, and examined with the **high power.**

THE PROSTATE GLAND.

The **prostate gland** is a compound tubular gland. The alveoli are embedded in a mass of smooth muscle and connective tissue intermingled, the former being greater in amount. The **excretory ducts** of the gland open into the floor of the urethra as it passes through the gland.

The **alveoli** are lined with simple cylindrical epithelium. In some alveoli a pseudostratified epithelium is found. This epithelium is, in many alveoli, thrown up into ridges.

In the old man many of the alveoli contain concentrically laminated bodies, the **corpora amylacea.** These bodies are comparatively few in the prostates of young men.

Practical Study.

Small pieces of the human prostate gland are fixed in formalin-Müller's fluid. After hardening in alcohol they are embedded in celloidin. Sections are stained **double** and mounted in **balsam.**

The prostates from both an old and a young man are to be used.

Prostate of a Young Man. Low Power.—Observe the irregular-shaped alveoli and their lining epithelium. Note the large size of the septa between the alveoli, and that the septa are composed chiefly of smooth muscle. Select a transverse section of an alveolus and turn on the

High Power.—Observe the epithelium of the alveolus, the sections of the ridges of the epithelium, which appear as projecting masses of epithelial cells. Note the structure of the septa between the alveoli.

Make a drawing of a single alveolus.

Prostate Gland of an Old Man.—Sections are to be stained **double** and mounted in **balsam.**

Low Power.—Search for an alveolus containing one of the **corpora amylacea** and turn on the

High Power.—Note the concentric lamellæ of the body and that it nearly fills the lumen of the alveolus, flattening out the lining epithelium. Note that the amount of smooth muscle tissue may show an increase.

Make a drawing of the alveolus and the contained **amyloid body.**

THE PENIS.

The penis consists of three cylindrical-shaped masses of **erectile tissue,** which are surrounded by loose connective tissue and skin.

The erectile tissue of the two dorsal masses, the **corpora cavernosa** and the **corpus spongiosum,** containing the **urethra,** which lies below and between the two former, are surrounded by a dense connective-tissue sheath, the **tunica albuginea.**

Erectile Tissue.—Erectile tissue consists of **septa** and **trabeculæ** composed of smooth muscle and connective tissue. These septa and trabeculæ enclose irregular-shaped, communicating channels, the **cavernous channels.** These channels are lined with a single layer of endothelial cells, and they communicate with the blood vessels of the penis. When the organ is in a state of erection the cavernous channels are filled with blood; otherwise they are in a state of collapse. The arteries of the corpora cavernosa have a very thick muscular coat. They run in the trabeculæ, and in the septa break up into a plexus of capillaries. A few of the arteries open into the cavernous channels, while others form a rich capillary network beneath the tunica albuginea, which is connected with a deeper venous network that also connects with the cavernous channels. There is also a direct anastomosis between the arterial and venous capillaries. By this arrangement the blood current may pass into the capillaries or, under certain conditions, into the cavernous channels, dilating them and causing the erection of the organ.

Urethra and Corpus Spongiosum.—The **urethra** is a tubular canal, the greater portion of its length lying in the **corpus spongiosum.** It is divided into the **prostatic, membranous,** and **spongy** or **penile portions.** It is lined with a **mucous mem-**

brane and surrounded by a connective-tissue layer, the **submucosa**, and in certain portions this is in turn surrounded by a **muscular layer.**

The **prostatic portion** has a distinct muscular layer divided into an internal longitudinal and external circular. It is composed of smooth muscle tissue. This portion of the urethra is embedded in the prostate gland.

The **membranous portion** extends from the prostate gland to the commencement of the corpus spongiosum. It is surrounded by the muscular layer.

The **penile** or **spongy** portion extends through the entire length of the corpus spongiosum. It terminates at the **glans penis** by an opening, the **meatus.** As it passes through the glans penis it becomes somewhat dilated, forming the **fossa navicularis.**

The coats of the penile portion, except at its entrance into the corpus spongiosum, consist of but two layers, the **mucous membrane** and the **submucosa.** The muscular layer of the deeper portions extends into the corpus spongiosum, but soon runs out.

The **mucous membrane** is thrown up into longitudinal folds when the urethra is collapsed. Its **stroma** is composed of fine connective-tissue fibres, rather rich in cells, and merges off into the submucosa.

The **epithelium** covering the surfaces of the mucous membrane varies in type in the different portions of the urethra.

In the **prostatic portion** it is stratified squamous, which changes into stratified cylindrical in the **membranous portion.** In the **penile portion** it is simple cylindrical or, according to some authors, pseudo-stratified, with two or three rows of nuclei. From the **fossa navicularis** on to the **meatus** it is stratified squamous.

Glands of Littré.—Are mucous glands of the compound tubular type. They are found distributed along the entire length of the penile portion of the urethra. Their alveoli are situated in the submucosa and their ducts pass to the surface of the mucous membrane.

The **submucosa** consists of rather coarse connective-tissue fibres, with but few cells, and it merges into the stroma of the mucous membrane. It contains in its outer portion a dense network

of large veins, the **erectile veins**, which form a species of erectile tissue.

The **erectile tissue** of the corpus spongiosum is in structure the same as that of the corpora cavernosa.

The **glans penis** is covered externally with stratified squamous epithelium, which is continuous with that lining the **prepuce**. At the base of the **glans penis** (the corona) there are a few sebaceous glands, the **glands of Tyson.**

PRACTICAL STUDY.

Corpus Spongiosum and Urethra.—The corpus spongiosum and the contained urethra is dissected away from the corpora cavernosa of the human penis. It is then cut into pieces about half an inch long and fixed in formalin-Müller's fluid; after hardening in alcohol, embedded in celloidin and transverse sections made.

The sections are stained **double** and mounted in **balsam.**

Naked Eye.—Note the general shape of the corpus spongiosum and the position of the urethra, which appears as an irregular slit-like opening.

Make an outline sketch of the specimen.

Low Power.—Observe the **urethra**, its lumen, and the folds of the mucous membrane; also the **erectile tissue**, its **cavernous channels**, and its **tunica albuginea.**

Make an outline drawing of one-half of the section, showing the **urethra**, its folded **mucous membrane**, the **submucosa**, and the **erectile tissue** with the **tunica albuginea.**

High Power.—Observe the **mucous membrane.** Note the lining of epithelial cells, the character of the stroma; the submucosa and its **erectile veins.** Next observe the structure of the **erectile tissue.** Note the **cavernous channels** lined with a single layer of endothelium. In some places these channels may be collapsed, in others partially dilated and containing blood cells. Next note the structure of the **septa**, the amount of smooth muscle, and the plane of the section through the smooth muscle cells. Also note the arteries with their thick middle coat.

Find a **gland of Littré**; note its situation and the structure of its alveoli. See if a duct of the gland shows in your specimen.

Make the following drawings:

A. Of a section through the mucous membrane of the urethra and submucosa, showing all details of structure.

B. Of the erectile tissue, showing all details of structure.

THE FEMALE GENITAL ORGANS.

THE OVARY.

The ovaries are oval-shaped bodies flattened from above downward. They average 1½ inches in length, 1 inch in width, and one-half an inch thick.

They are situated on either side of the uterus, their base, or **hilum**, being attached to the posterior surface of the broad ligament of the uterus. The inner end of each ovary is attached to the horn of the uterus by the **ovarian ligament.** The surface of the ovary is covered with a single layer of cylindrical epithelium, the **germ epithelium.**

The ovary consists of connective tissue, the **stroma**, and the glandular elements, the **Graafian follicles.**

It is divided into two zones, the **cortex** and the **medulla.**

The **cortex** contains the **Graafian follicles** embedded in the stroma. The free surface of the organ is covered with a single layer of cylindrical epithelial cells, the **germ epithelium** Directly under the germ epithelium is a condensed zone of the stroma known as the **tunica albuginea**, which merges off into the stroma.

The **medulla** occupies the central portion of the ovary and is composed of numerous convoluted blood vessels embedded in a mixture of smooth muscle and connective-tissue elements.

The **stroma** consists almost entirely of spindle-shaped, flattened, and irregular branching cells. The stroma also contains numerous small-sized blood vessels.

Graafian Follicles.—The Graafian follicles are vesicular cavities, of various sizes and in various stages of development, situated in the cortical zone. Each follicle contains a highly differentiated cell, the **ovum.**

Each follicle is surrounded by a distinct wall, the **theca folliculi.** It is divided into two layers—an **external** dense one, the **tunica fibrosa**, which contains numerous blood vessels; and an inner one,

tunica propria, which contains numerous small blood vessels. A third layer, the **membrana propria** (Waldeyer), has been described.

The **theca folliculi** is lined with several layers of large, cuboidal-shaped cells, the **follicular epithelium** or **membrana granulosa.**

At one side of the mature follicle this epithelium becomes heaped up into a mass forming the **germ hill**, or **discus proligerus.** The **ovum** is embedded in this mass of cells and is surrounded by a layer of high cylindrical cells, the **corona radiata.**

The **cavity** of the follicle, or **antrum**, is filled with an albuminous fluid, the **liquor folliculi.**

The follicle, as it matures, approaches the surface and comes in contact with the tunica albuginea; the latter, with the theca folliculi, becomes thinned. This region is known as the **stigma**, and it is at this point that the follicle finally ruptures.

An increase in pressure within the follicle causes its rupture, and the ovum escapes surrounded by the cells of the discus proligerus. It is taken up by the **fimbriated** end of the Fallopian tube and passes on to the cavity of the uterus.

The Ovum.—The ovum is a typical cell, spherical in shape, and in the human subject measures from 0.22 to 0.32 mm. in diameter. It is surrounded by a cell membrane, the **zona pellucida.**

The **cell body**, or **vitellus**, consists of two substances—the **network**, which is somewhat denser at the periphery and in the neighborhood of the nucleus; and highly refractive, **oval-shaped bodies** embedded in the meshes of the network.

The **nucleus**, or **germinal vesicle**, is surrounded by a distinct membrane. The intranuclear network is scanty and contains but little chromatin. It contains a **nucleolus** or **germinal spot.** In some cases two or more nucleoli are present. They are now believed to be nodal thickenings of the chromatin.

Corpus Luteum.—After the discharge of the ovum the cavity of the follicle becomes filled with blood. Then a proliferation of the cells of the tunica interna of the theca folliculi takes place. These cells gradually penetrate the blood clot and are accompanied by thin septa of connective tissue containing blood vessels.

The proliferating cells from the theca folliculi contain large epithelioid cells, the cytoplasm containing many granules. These are the **lutein cells.** It is to these cells that the color of the corpus luteum is due. The proliferating layer of cells becomes folded in, capillaries from the blood vessels in the septa penetrate between the lutein cells, and gradually the folded membrane encroaches on the blood clot, which becomes absorbed. Finally the interior of the corpus luteum becomes filled with connective tissue, which gradually becomes absorbed and all traces of the corpus luteum become obliterated.

There are two kinds of corpora lutea, the **true** and the **false.**

When the ovum that has been discharged from a follicle is not fertilized, the corpus luteum formed is known as a **false** one, **or corpus luteum** of **menstruation.** If the ovum be fertilized, the **true corpus luteum,** or **corpus luteum** of **pregnancy,** is formed. This remains, at least, until the end of pregnancy, and then becomes absorbed.

Development of the Graafian Follicle.—During fœtal life some of the cells of the germ epithelium become differentiated. They enlarge, become spherical in shape, forming the **primitive ova.** These cells are carried down into the stroma of the ovary by cord-like masses of the germ epithelium—**Pflüger's plugs.** These plugs contain several of the primitive ova. The plugs are cut off from the germ epithelium and become surrounded by stroma and are now known as **egg nests.** The epithelial cells surrounding the ova rapidly proliferate, and an ovum surrounded by a layer of cells becomes cut off from the egg nest. This mass becomes spherical in shape, the ovum being in the centre, and is surrounded by a layer of flat or cuboidal cells. This is a **primitive Graafian follicle.** At the time of birth the ovary consists almost entirely of primitive follicles, and it is estimated that each ovary contains about 36,000.

The growth of the follicle takes place by means of the proliferation of the epithelial cells surrounding the ovum. These increase until the ovum becomes surrounded by several layers of cells. The ovum, which at first occupied the centre of the mass of cells, assumes an eccentric position. A fissure appears among the epithelial cells, which gradually enlarges into a cavity, the **antrum.**

This becomes filled with a fluid, the **liquor folliculi.** The follicle increases in size through proliferation of its cells; the ovum, embedded in a mass of cells, the **discus proligerus,** becomes pressed more and more to one side; the size of the antrum increases, and the stage of a **mature Graafian follicle** is reached. During the growth of the follicle the stroma surrounding it becomes differentiated into the **theca folliculi.**

Not all of the primitive follicles reach maturity; many become destroyed by a process known as *atresia of the follicle.* This process begins in the ovum and extends to the follicular epithelium. Both become destroyed by an albuminous degeneration, and the follicle is converted into fibrous tissue, forming the corpus albicans, similar to that of the corpus luteum.

Blood Vessels.—The blood vessels enter the ovary at the hilum and pass up into the medulla, assuming a tortuous course. Branches are given off which penetrate the cortical zone and break up into a capillary network under the tunica albuginea. The theca folliculi is also abundantly supplied with a network of vessels which break up into capillaries in its inner layer.

The nerves follow the course of the blood vessels, but seldom, it is believed, penetrate into the interior of a Graafian follicle. Ganglion cells are said to occur in the medulla near the hilum.

The **parovarium,** or the **organ of Rosenmüller,** is a scattered group of tubules lying in the mesosalpinx between the ovary and Fallopian tube. They are blind tubules, lined with cylindrical epithelium which may be ciliated or non-ciliated.

The **epoöphoron** consists of tubules similar to those of the parovarian and lying in the broad ligament near the hilum of the ovary. These tubules connect with **Gärtner's duct.**

Both of these structures are remains of the **Wolffian body,** a fœtal structure, from which the ovary is developed.

Practical Study.

Ovary of a Child at Birth.—The ovary of a child is removed, post mortem, with the greatest of care, so that the germ epithelium will not be brushed off in the manipulations. It is fixed in a large quantity of Zenker's fluid and hardened in alcohol. Embed in

celloidin and make transverse sections of the organ. These are stained **double** and mounted in **balsam.**

Low Power.—(a) Observe the **germ epithelium** on the surface and note its continuation with flattened mesothelial cells of the broad ligament. **(b)** The **cortical zone** of the ovary filled with the **primitive Graafian follicles.** In most sections the **hilum** of the ovary and its attachment to the broad ligament will show, and possibly sections of some of the tubules of the **epoöphoron.**

Make an outline drawing of the entire ovary and its attachment to the broad ligament.

High Power.—Select a section of a **primitive Graafian follicle.** Observe the ovum in its centre, and that it is surrounded by a single layer of cuboidal or flattened cells.

Make a drawing of a single follicle showing all details of structure.

Adult Ovary.—The ovary of an adult cat or dog is to be carefully removed, care being taken not to touch its surface, as the germ epithelium is easily rubbed off in the fresh state. The ovaries of animals have to be used, as the human ovaries obtained at autopsies often show pathologic changes, and those taken from women of middle age show but few follicles.

The ovary is fixed, in bulk, in Zenker's fluid and hardened in alcohol. Embed in celloidin and make longitudinal sections. The sections from the central portion of the ovary are the only ones that will show topography. The sections are stained **double** and mounted in **balsam.**

Low Power.—Identify the **germ epithelium,** the **tunica albuginea,** the **cortical zone** and its **Graafian follicles,** the **medulla** and its tortuous blood vessels.

Make a drawing of a section through the ovary showing the different zones.

High Power.—Observe the **germ epithelium,** the **tunica albuginea,** the various-sized **follicles** in the **cortical zone** and the **stroma.** Select a **young follicle,** note its *theca folliculi, the follicular epithelium, the ovum,* and the commencing formation of the *cavity of the follicle or antrum.*

Next select a more **mature follicle.** Note the larger size of the

antrum, the *follicular epithelium* and *ovum,* and the commencement of the formation of the *germ hill,* or *discus proligerus.*

Next select a **mature follicle** and proceed as above.

Make a drawing of the germ epithelium and the underlying tunica albuginea, and of each of the above-described forms of the Graafian follicles.

FALLOPIAN TUBE.

The **Fallopian tube,** or **oviduct,** arises from the horn of the uterus and lies in the upper folded edge of the broad ligament.

It is divided into three parts: **isthmus,** or **uterine end,** about one-third of the length of the tube; the **ampulla,** a little more than the middle third; and the **fimbriated end.**

The **isthmus** begins at the horn of the uterus as the ostium uterinum; it runs a moderately straight course and is small in diameter.

The **ampulla** is twice the diameter of the isthmus; it has a curved course.

The **fimbriated end** widens out into a cone-shaped body, and its end is broken up into a series of **fimbriæ,** one of which, the **fimbria ovarica,** is attached to the external surface of the outer end of the ovary.

The **wall** of the Fallopian tube consists of three layers, the **outer** or **serous,** the **middle** or **muscular,** and the **inner** or **mucous membrane.**

The **serous coat** is formed by the broad ligament and surrounds only about three-quarters of the tube. It consists of loose connective tissue, the free surface of which is covered with a layer of mesothelial cells.

The **muscular coat** consists of smooth muscle laid down in two layers, an inner circular and an outer longitudinal layer. At the outer end of the tube these layers are not sharply defined.

The **mucous membrane** consists of a stroma of fine connective-tissue fibres containing many cells, and is covered with a layer of simple, ciliated cylindrical epithelial cells, the cilia of which whip from the fimbriated end toward the uterus. A thin muscularis mucosæ is sometimes present. The mucous membrane is thrown up into a series of longitudinal, narrow folds. In the isthmus

these folds are low; they gradually increase in height, and when the fimbriated end is reached they become very complicated owing to the secondary folds which are given off from the sides of the primary ones. The **lumen** of the Fallopian tubes becomes continuous with the pelvic cavity at the fimbriated end.

The blood vessels are abundant in the mucous membrane, forming a dense capillary network. The larger vessels run along the base of the folds of the mucous membrane.

Practical Study.

Human Fallopian Tube.—A human Fallopian tube, obtained post mortem or at an operation, is fixed in formalin-Müller's fluid and hardened in alcohol. After embedding in celloidin, *transverse* sections are made through its various divisions, stained **double,** and mounted in **balsam.**

Low Power.—Observe the various coats of the tube, especially the **mucous membrane,** and the appearance of its folds in the various sections.

Make an outline drawing of the sections showing the various coats.

High Power.—Observe the structure of the serous coat, the muscular coat, and the mucous membrane. Note the stroma of the latter, the primary and secondary folds, and the ciliated epithelium covering the surface (often, in human specimens, the cilia do not show on account of postmortem changes).

Make a drawing of a single fold of the mucous membrane showing all details of structure.

THE UTERUS.

The **uterus** is a pear-shaped, muscular organ flattened in its anterior posterior diameter. It is divided into the **body** and **neck** or **cervix.** Its external surface is covered with a serous membrane, a reflexion of the pelvic peritoneum.

The **cavity of the uterus** is divided into that of the body and that of the cervix. It is lined with a mucous membrane.

The **muscular wall** of the uterus is composed of smooth-muscle tissue laid down in three layers, an inner longitudinal, a middle, somewhat circular, and an external longitudinal. The middle

layer contains the principal blood vessels. The muscle cells differ slightly from those found in other organs. They are fusiform in shape, with blunted ends, the nucleus being oval in shape.

The **mucous membrane** of the cavity of the body is about 1 to 1½ mm. in thickness. Its surface is comparatively smooth and is covered with a single layer of ciliated cylindrical epithelium, the cilia of which whip from within outward. Its stroma consists of elongated cells with oval nuclei; the cells have numerous processes which join with those of neighboring cells, forming a network the meshes of which are filled with spherical-shaped cells resembling lymph cells and leucocytes.

Embedded in the stroma are **forked tubular glands**, the surface portions of which run a rather straight course, while the deeper portions run an irregular one. These glands open on the surface of the mucous membrane, and are lined with a single layer of ciliated cylindrical epithelial cells which are continuous with those on the surface of the mucous membrane. These glands have a membrana propria made up of flattened connective-tissue cells.

The above description applies to the mucous membrane of the resting organ only. During menstruation and pregnancy marked changes take place.

At the **menstrual period** the mucous membrane becomes much thickened and its surface irregular. The cellular elements proliferate, the tubules increase in size, and the capillaries and veins become very much distended, whereby the blood supply is increased.

This first stage is followed by a disintegration of the superficial portion of the mucous membrane, with an escape of blood from the ruptured blood vessels. The surface epithelium and a portion of the surface of the stroma is thrown off.

Regeneration now commences. The congestion disappears, the stroma is regenerated, and the surface epithelium restored from a growth of that lining the tubules.

The **mucous membrane** of the **cavity of the cervix** is elevated, anteriorly and posteriorly, into large, longitudinal folds which give off lateral folds. These folds are known as the **plicæ palmatæ.** The glands are forked tubular ones lined with cylindrical ciliated epithelium which extends over the surface of the mucous mem-

brane. In addition to the glands the cervical mucous membrane contains short **crypts** with lateral branches. The lumen of the crypts is wider than that of the glands, and the epithelium higher. The cylindrical epithelium of the cervical, as it approaches the external os, changes into stratified squamous and becomes continuous with that covering the external surface of the cervix. The mucous membrane of the cervix often contains retention cysts, the **ovulæ Nabothi.**

The uterus receives its blood supply from both the uterine and ovarian arteries. Branches enter the uterus along its sides and pass into the middle muscular layer and give off branches. Some of these pass into the mucosa, where they break up into capillaries which surround the glands and also form a network beneath the surface epithelium. The veins form a plexus in the deep portion of the mucosa; branches pass outward to the middle muscular layer and finally empty into the uterine and ovarian veins.

The lymphatics, which are numerous, begin as clefts in the mucous membrane; the lymph capillaries, joining, form lymph vessels which pass outward to the middle muscular layer, forming a plexus the vessels of which empty into the larger lymph vessels of the serous layer.

The uterus has numerous nerve fibres of both the medullated and non-medullated variety. The latter terminate in the muscle; the former have been traced into the mucosa.

Practical Study.

Human Uterus.—The human uterus is cut, transversely, into pieces about one-quarter of an inch thick and fixed in Zenker's fluid and then hardened in alcohol. These pieces are then trimmed up into rectangular-shaped blocks, leaving a margin of about one-eighth of an inch of the muscular tissue surrounding the uterine canal. Embed these blocks in celloidin and make transverse sections through them.

The sections are stained **double** and mounted in **balsam.**

Transverse Section through the Body of the Uterus. Low Power.—Observe the opening in the central portion of the section, the **cavity** of the uterus, which is surrounded by the **mucous mem-**

brane containing the tubular glands. Outside of the mucous membrane is the **muscular tissue.**

High Power.—Observe the layer of cylindrical epithelium covering the surface of the mucous membrane and dipping down into the funnel-shaped mouths of the glands. Owing to postmortem changes the cilia of the cells may not show and the surface cells may be more or less removed.

Next note the tubular glands; observe that, owing to their irregular course, no section of an entire gland can be seen, but their general course can be determined by the series of interrupted sections. Note that the glands extend outward nearly to the muscular coat. Next observe the structure of the **stroma.** Finally pass out to the **muscular tissue** and note its structure.

Make a drawing of a section through the mucous membrane showing all details of structure.

Transverse Section through the Cervix of the Uterus.—Proceed in the same manner as with the transverse section through the body.

THE VAGINA.

The **wall** of the vagina is composed of three coats: the **external** or **fibrous,** the **muscular,** and the **lining mucous membrane.**

The **fibrous coat** is formed of dense connective tissue containing a large number of elastic fibres. It is connected with adjacent structures by loose connective tissue.

The **muscular coat** consists of smooth-muscle tissue with an indistinct separation into an inner circular and an outer longitudinal layer. The inner or circular layer is poorly developed and may be absent.

The **mucous membrane** is covered with stratified squamous epithelium, the surface cells being exceedingly thin, and they rest on a stroma of connective tissue from which numerous papillæ are given off. The stroma consists of interlacing bundles of fibrillated fibres intermingled with a large number of coarse elastic fibres. Considerable diffuse lymphatic tissue is found in the mucosa, and it sometimes occurs in the form of solitary nodules. The mucosa rests on a submucosa composed of coarse connective-tissue

fibres. Glands have been described as occurring in the vagina, but this is doubted by most observers.

Practical Study.

Human Vagina.—Pieces of the human vagina are pinned out on sheet cork, its fibrous coat down, and fixed in formalin-Müller's fluid, then hardened in alcohol and embedded in celloidin.

Sections are made at a right angle to the surface of the mucous membrane, stained **double,** and mounted in **balsam.**

Low Power.—Identify the coats and make an outline drawing showing the relations to each other.

High Power.—Observe the structure of the **mucous membrane,** and make a drawing of it showing all details of structure.

THE MAMMARY GLAND.

The mammary gland is a compound tubular gland made up of from fifteen to twenty **lobes,** which are again made up of **lobules.** The lobes are separated by loose connective tissue which sends thin septa into them and between the lobules. Each lobe has **an excretory duct,** which sends branches to the lobules; these **lobular ducts** break up into branches, the **terminal ducts,** around which are grouped the **alveoli.** The excretory duct enters the **nipple,** passes up through it, and opens on its surface. At the base of the nipple these ducts dilate into irregular sacs, the **ampullæ,** which serve as storage reservoirs for the milk.

From birth until puberty the gland is growing constantly. The male and female glands up to the twelfth year are identical in structure. After this age, in the female, they continue to develop, while in the male they atrophy. They do not reach their full development in the female until the last month of pregnancy, and they become functionally active soon after the birth of the child.

There are marked differences in the structure of the **active** and the **inactive** gland.

Active Mammary Gland.—In the active gland the **tubular alveoli** are lined with a single layer of low cylindrical or cuboidal

epithelium resting on a homogeneous **membrana propria.** The appearance of these cells varies according to their state of activity. The **resting cell** is somewhat cuboidal in shape, with a granular cytoplasm. At the commencement of **activity** the cells increase in length; fat granules appear in the distal end of the cytoplasm; these increase in size and, fusing, form droplets; these droplets, fusing, form a large globule of fat, which appears in the free end of the cell. This is discharged from the cell, and the cell is regenerated from the unchanged cytoplasm and nucleus. How often the cells are regenerated is not known, but it is certain that cells are destroyed, in the process of secretion, and replaced by new ones.

The **ducts** consist of a *membrana propria* and are lined with a single layer of *epithelial cells* of the cylindrical type. The epithelium in the large excretory ducts is high. As the ducts grow smaller the height of the cell decreases, and when the *terminal ducts* are reached it becomes low or even flat.

Not all of the alveoli of a lobule are **active** at the same time. Each lobule shows a certain proportion of **inactive** alveoli; these are lined with a single layer of cuboidal granular cells.

Inactive Mammary Gland.—There is a marked difference in the appearance of inactive from the active gland. The inactive gland consists of a series of ramifying ducts, around the terminations of which are grouped the collapsed alveoli, lobules which are separated from each other by masses of rather dense connective tissue.

Milk.—The milk consists of **fat globules,** from 2 to 5 μ **in** diameter, and surrounded by an albuminous membrane which prevents their coalescence. They are suspended in a clear fluid, the **milk plasma.** Before parturition the milk contains rather large nucleated cells with fat globules in the cytoplasm, the **colostrum corpuscles.** These colostrum corpuscles are considered by some as leucocytes that have undergone fatty degeneration; others, that they are cast-off epithelial cells in a state of fatty degeneration.

Practical Study.

Active Mammary Gland.—Small pieces of the mammary gland

of a nursing woman are fixed in formalin-Müller's fluid, then hardened in alcohol. They are embedded in celloidin with the same care as adult fat tissue (see page 68). Sections are stained **double** and mounted in **balsam.**

For demonstrating the fat granules and fat drops in the cells, exceedingly small pieces of the gland are treated with osmic acid for twenty-four hours, then embedded and sections made. The sections are stained with hæmatoxylin and mounted in pure glycerin.

Low Power.—Observe the outlines of the **lobules** marked out by fine **septa** of connective tissue; the **alveoli** of the lobules; the **ducts** lined with a single layer of cells.

Make an outline drawing of a portion of a lobule showing its structure.

High Power.—A. Select an **active alveolus.** Observe the membrana propria and the lining epithelial cells. Note the various conditions of activity of the cells.

Make a drawing showing all details.

B. Select a **resting** alveolus and note the characteristics of the cells.

Make a drawing showing all details.

C. Select a *transverse section* of an **interlobular duct** and of one of the **large ducts.** Note their structure.

Make a drawing of these ducts.

Inactive Mammary Gland.—Pieces of an inactive mammary gland are prepared in the same manner as the active gland.

Low Power.—Observe the sections of the ducts and the alveoli grouped around them. Note the amount and character of the connective tissue.

Make a drawing of at least one duct and its alveoli.

THE SKIN AND ITS APPENDAGES.

The skin is composed of a connective-tissue layer, the **derma, corium,** or **true skin,** and an epithelial layer, the **epidermis.** It is attached to the underlying structures by a rather loose connective-tissue layer, the **subcutaneous tissue.**

The appendages of the skin are the **nails, hairs,** and **sweat** and **sebaceous glands,** all of which are derived from the epidermis.

THE SKIN.

Epidermis.—The epidermis is composed of stratified epithelium, which is divided into two distinct strata, the deep or **stratum mucosum** (stratum Malpighii, stratum germinativum), and the superficial or **stratum corneum** (horny layer). In regions where the epidermis is very thick (palms of the hands and soles of the feet) substrata become developed. These are the **stratum granulosum** of the mucous strata and **stratum lucidum** of the stratum corneum.

The cells of the **stratum mucosum,** on account of their shape, may be divided into three zones: the deep, consisting of a single layer of cylindrical cells with oval nuclei; the middle, consisting of several layers of polygonal-shaped cells; and the superficial, or **stratum granulosum,** consisting of two or three layers of flattened cells.

The deep layer of cells rests on the basal membrane and follows the outlines of the papillæ of the derma and the spaces between them.

The cells of the middle zone consist almost entirely of the **spine** or **prickle cells,** and for this reason this zone has been called the **stratum spinosum.** These cells are large, of a polygonal shape, and contain a spherical-shaped nucleus. The processes—spines or **intercellular bridges**—are given off from the cell body

and pass across minute channels between the cells. These channels communicate with the lymphatics of the derma.

The cells of the superficial zone, or **stratum granulosum,** appear, in section, fusiform in shape, and contain an oval-shaped nucleus. The cytoplasm is coarsely granular. The granules (keratohyalin granules) stain with the nuclear dyes.

The **stratum lucidum** is a narrow, homogeneous layer outside of the stratum granulosum.

The **stratum corneum** consists of several layers of flattened, hornified cells without nuclei. Its thickness varies in different regions; sometimes it is very thin, consisting of but a few layers of cells. In the palms of the hands and the soles of the feet it is exceedingly thick. The surface cells are continually being cast off and replaced by the underlying cells. Cell proliferation takes place rapidly in the deeper layers of the stratum mucosum; the young cells, passing outward through the different strata, undergo various stages of differentiation and are finally cast off from the surface of the skin as desiccated cells.

Derma, or True Skin.—The derma is a connective-tissue layer and is divided into two sublayers: the deep, or **pars reticularis,** and the superficial, or **pars papillaris.** There is no sharp line of demarcation between them; they gradually blend one with the other.

The **pars reticularis** consists of bundles of fibrillated fibres arranged in the form of a coarse-meshed network, the bundles running, for the most part, parallel with the surface of the skin, and they are surrounded by a network of coarse elastic fibres.

In the **pars papillaris** both kinds of fibres are finer than those of the pars reticularis, though the arrangement is somewhat similar, and it has a denser appearance. The conical-shaped **papillæ** are given off from the outer surface of this layer, and they project into the stratum mucosum of the epidermis. These papillæ end in one or more blunted points and are classified as **simple** and **compound.** Some of the papillæ contain blood vessels alone, others a special form of nerve termination, the **tactile corpuscles,** and are known as **vascular** and **nervous papillæ.** The surface of the pars papillaris is covered by a thin basal membrane.

The **subcutaneous tissue** is composed of numerous vertical bands of connective tissue, the retinaculæ cutis, which join the

pars reticularis to the superficial fascia and other underlying structures. These bands enclose masses of fat cells, the **panniculus adiposus.** They contain blood vessels, nerves, and the ducts of the sweat glands.

Smooth muscle occurs in the skin in the form of the erector pili muscle attached to the hair follicle, and as the tunica dartos in the scrotum. In the face and neck striated muscle fibres are found extending into the derma.

The color of the skin is due to the pigmentation of deeper layers of the epidermis, and in certain localities also to the pigmentation of the connective-tissue cells of the derma. In some of the colored races the pigmentation of the cells of epidermis may extend entirely through it, though it is usually confined to the deeper cells alone.

Sweat Glands.—The sweat glands are coiled tubular glands, the coiled portion being situated usually in the subcutaneous tissue, though it may occur in the deeper portion of the pars reticularis of the derma. The excretory duct passes up through the derma nearly straight, enters the epidermis between two papillæ, and then assumes a spiral course to the surface. The membrana propria is thin and structureless. The coiled or secretory portion is lined with a single layer of cuboidal-shaped cells, with round or oval nuclei and granular cytoplasm. In this portion of the gland there is a thin longitudinal layer of smooth muscle between the cells and the membrana propria. In the duct portion of the gland there are two layers of cells; these, together with the membrana propria, cease as soon as the horny layer of the epidermis is reached, and the duct continues as a channel through this layer.

Nerve Terminations.—Two special forms of nerve terminations are found in the skin, the **tactile corpuscles,** or **Meissner's corpuscles,** and the **Pacinian bodies,** or **Vater's corpuscles.**

The **tactile corpuscles** are oval-shaped and are enclosed by a thin connective-tissue capsule in which there are oval-shaped nuclei. The medullated nerve fibre, upon reaching the base of the corpuscle, loses its medullary sheath, and the naked axis cylinder, entering, makes a number of spiral turns and usually ends in a number of branches. These corpuscles are found in the nervous papillæ of the derma.

The **Pacinian bodies** are of a somewhat elongated oval shape,

being composed of a series of layers, from twenty to sixty, of fibrous tissue grouped in a concentric manner around a central axis. Both surfaces of the lamellæ are covered by a single layer of flat cells. The axis of the body is occupied by the core, made up of semi-fluid granular material. A medullated nerve enters the base of the body, and when the core is reached the naked axis cylinder passes into it, where it may divide into from two to four branches, or it may continue through the core to its distal end and then break up into from two to five branches. In either case the branches end in large, irregular end discs. The Pacinian bodies are in the deep portion of the derma and in the subcutaneous tissue.

PRACTICAL STUDY.

Section of Skin from the Finger Tip.—The palmar surface is removed from the distal phalanx of the finger, including the tissue down to the bone. The specimen is fixed in formalin-Müller's fluid, hardened in alcohol, and embedded in celloidin. Transverse sections are made, stained **double,** and mounted in **balsam.**

Low Power.—Observe the three layers of the skin: the **epidermis,** the **derma,** and the **subcutaneous tissue.**

Identify the **stratum corneum,** the **stratum lucidum,** and the **stratum mucosum** of the epidermis; the **derma** with its **papillæ,** and the **subcutaneous tissue** with its panniculi adiposi (fat lobules) surrounded by the strands of connective tissue.

Note the sections of the **sweat glands** in the subcutaneous tissue, and the transverse or longitudinal sections of the **Pacinian bodies.** Also note the corrugations on the surface of the stratum corneum, sections of the ridges of the epidermis, and the corkscrew channel of the duct of the sweat gland.

Make a drawing showing the various layers of the skin.

High Power.—1. Note the structure of the stratum corneum; the stratum lucidum; the stratum mucosum, its deep layer of cylindrical cells, the middle layers of polygonal cells with their intercellular bridges (spine or prickle cells); and the stratum granulosum.

2. Note the structure of the derma and its division into the deep layer, or pars reticularis, and the superficial layer, or pars papillaris. Also note the two kinds of papillæ: the vascular, contain-

ing a capillary network, and the nervous, containing a tactile corpuscle.

3. Note the structure of the subcutaneous tissue; the sweat glands, the panniculi adiposi, and the Pacinian bodies.

Make the following drawings showing all details of structure: A section through the epidermis; a vascular papilla, a nervous papilla; a section of the coiled portion of the sweat gland; a section of a Pacinian body, and of the prickle cells of the stratum mucosum.

Negro's Skin.—Pieces of the skin of the negro are prepared in the same manner as the finger tip.

Sections are stained **double** and mounted in **balsam.**

High Power.—Note the situation of the pigmented cells.

THE NAILS.

The nails are a modification of the epidermis. They are divided into the **free edge, body,** and **root.** The body, which is arched, lies on the **nail bed or matrix;** the root is embedded in a groove at the posterior end, the **sulcus.** The nails are bounded on either side by a fold of the skin, the **nail wall.** Between the nail wall and matrix there is a depression, the **nail groove.**

The **nail bed,** or **matrix,** consists of a series of high, parallel ridges of the derma, these ridges running parallel to the long axis of the nail. They are covered with stratified epithelium which corresponds to the stratum mucosum.

The **nail** consists of horny epithelium and corresponds in structure to the stratum corneum, except that the cells are nucleated.

Practical Study.

Transverse Section of the Finger Nail.—The distal phalanx is removed from the hand of a new-born child and fixed in formalin-Müller's fluid, hardened in alcohol, and embedded in celloidin. Transverse sections are made through the specimen, stained **double,** and mounted in **balsam.**

Low Power.—Note the **nail** resting on its **nail bed;** also note the **nail wall** and **nail groove.**

Make a drawing of one-half of the nail and nail bed.

THE HAIR AND ITS FOLLICLE.

Hairs are found, with a few exceptions, wherever the skin exists, the quantity and arrangement differing in different regions. They are absent in the palms of the hands and soles of the feet, and the dorsal surface of the third phalanges of the fingers and toes. On the scalp they are arranged in groups; on the rest of the skin they usually occur as single ones. They are flexible and elastic.

A hair is divided into three portions: the **shaft,** which projects above the surface of the skin; the **root,** that portion embedded in the skin; and the **bulb,** the lower expanded extremity which rests on the hair papilla. Each hair is situated in a tubular depression, the **hair follicle,** which lies at an angle with the surface of the skin.

Structure of the Hair Follicle.—The hair follicle is divided into the **wall** and **root sheath.** The wall is made up of connective-tissue elements and is the analogue of the derma. The root sheath is composed of epithelial tissue and is the analogue of the stratum mucosum of the epidermis.

The **wall of the hair follicle** is divided into three layers: the **outer** or **fibrous,** the **inner** or **vascular,** and the **vitreous membrane.**

The **outer layer** is composed of rather coarse connective-tissue fibres poor in cellular elements. They run somewhat longitudinally and contain large blood vessels.

The **inner or vascular** layer is composed of fine connective-tissue fibres which run in somewhat of a circular manner. The cellular elements are abundant. This layer contains a rich capillary network and small blood vessels.

The **vitreous membrane** is thin and homogeneous in structure and lines the interior of the follicle. It corresponds to the basal membrane of the derma.

The **root sheath** is composed of epithelial elements and is divided into the **outer root sheath** and the **inner root sheath.** The inner root sheath is subdivided into the **outer,** or **Henle's layer,** and the **inner,** or **Huxley's layer.**

The **outer root sheath** is made up of epithelial cells from the stratum mucosum. A row of cylindrical-shaped cells lies next

to the vitreous membrane; inside of these are several layers of polygonal-shaped cells of soft consistency but without intercellular bridges.

Henle's layer of the inner root sheath consists of a single layer of non-nucleated cells with clear cytoplasm. **Huxley's** layer is made up of two rows of nucleated cells with slightly granular cytoplasm.

At the bottom of the follicle the wall forms a conical-shaped mass, the **papilla,** which projects into the interior, and its upper portion is surrounded by the bulb of the hair. It contains a rich capillary network of blood vessels and many cellular elements.

The different layers of the root sheath are only well developed along the middle portion of the body of the hair. As the shaft and bulb of the hair are approached they merge into one another and finally become indistinct.

The **hair** consists entirely of epithelial cells, those of the body and shaft being horny. The cells are laid down in three layers, the **cuticle,** the **cortical substance,** and the **medulla.**

The **cuticle** is made up of a single layer of flat, transparent cells without nuclei, and they lap in a manner similar to that of shingles on a roof.

The **cortical substance** is made up of layers of elongated cells packed closely and they contain thin nuclei. These cells also contain pigment granules. At the bulb of the hair these cells are softer, but when the shaft is reached they have become horny.

The **medulla** does not extend the entire length of the hair, and in the thinner ones it is absent. It is made up of cuboidal-shaped cells with a finely granular cytoplasm. Some contain a nucleus, others do not.

A narrow band of smooth muscle, the **erector pili muscle,** is attached to each hair follicle. It arises in the superficial portion of the derma, on the side toward which the hair slopes; passing obliquely downward, it is inserted in the wall of the follicle at about the junction of its middle and lower thirds. The contraction of this muscle causes the hair to assume a perpendicular position—"stand on end." In its course the muscle passes around the base of the sebaceous gland, and in contracting compresses it, thereby aiding the gland to discharge its secretion.

The Sebaceous Glands.—The sebaceous glands are circular glands connected with the hair follicle, into which they pour their secretion. Sebaceous glands unconnected with the hair follicle are found in the glans penis (Tyson's glands), on the edge of the lips, labia minora, and in the eyelids (Meibomian glands).

They have a wide excretory duct, which opens into the mouth of the hair follicle, and around its deep portion are grouped a number of sac-like acini. The mouth of the duct is lined with stratified squamous epithelium, a continuation of the outer root sheath. As the acini are reached the epithelium becomes reduced to a single layer which is continuous with that lining the acini. The mouths of the acini are lined with cuboidal cells, which become spherical or polyhedral in the central portions. The cytoplasm of the cells of the central portion are filled with fat globules, which give it a reticular appearance. The nuclei are compressed and often distorted.

The secretion, the **sebum,** is a semi-fluid, oily substance containing many disintegrated cells.

Practical Study.

Sections of Hairs from Skin of Scalp.—Pieces of the human scalp are pinned out on sheet cork and fixed in formalin-Müller's fluid, hardened in alcohol and embedded in celloidin (see precautions to be taken under the preparation of adult fat).

Sections are made through the entire scalp and in a plane so as to cut the entire hair and follicle longitudinally. They are stained **double** and mounted in **balsam.**

Low Power.—1. Observe the **wall** of the **hair follicle** and the **papilla** projecting from its bottom; the **root sheath** and its division into **outer root sheath** and **inner root sheath**; the **hair** with its **bulb** resting on and surrounding the upper portion of the papilla; the **root** of the hair lying in the upper part of the follicle.

2. Observe the **erector pili muscle.** Note its point of origin in the derma and its insertion in the wall of the follicle.

3. Observe the **sebaceous gland** at the side of the follicle. Note its duct, the size and shape of the acini, and the relation of the erector pili muscle to its base.

Make an outline drawing showing all of the above parts.

NOTE that, in order to make this drawing, sections of various follicles may have to be selected, as it is almost impossible to find a hair and its follicle cut longitudinally throughout its entire length.

High Power.—1. Observe the structure of the **wall** of the **follicle,** its outer or **fibrous layer,** its inner or **vascular layer,** and the **vitreous membrane.**

2. Observe the structure of the **outer root sheath.** Note the cylindrical cells next the vitreous membrane, and the several layers of polygonal cells to the inside.

3. Observe the structure of the **inner root sheath**; its division into outer or **Henle's layer,** and inner or **Huxley's layer.**

4. Observe the structure of the **hair,** its medulla, its cortex, and its cuticle.

5. Observe the structure of the **bulb of the hair,** and the **papilla.** Note that at this part of the follicle the layers of the root sheath blend with each other.

6. Observe the structure of the **sebaceous gland.** Note the character of the epithelium lining the acini and duct.

Make the following drawings showing all details of structure: 1. Of a section through the hair, root sheaths, and wall of the follicle.

2. Of the bulb of the hair and the papillæ.

3. Of the sebaceous gland.

THE EYE.

The eyeball is a spherical-shaped organ, flattened somewhat in the antero-posterior direction. Its cavity contains partly formed and partly fluid elements. Posteriorly it is pierced by the optic nerve.

The wall of the eyeball consists of three coats: the *external,* or *sclera* (sclerotic, fibrous), a continuation of the dura mater of the brain—anteriorly it projects in a convex manner, forming the *cornea,* which is transparent; the *middle,* or *choroid* (tunica vasculosa, vascular layer), divided into the *choroid proper, ciliary processes,* and *iris;* and the *inner,* or *retina* (nervous coat). The *cavity* of the eyeball is divided by the *lens* into the *posterior chamber,* or *cavity of the vitreous humor,* and the *anterior chamber,* or *cavity of the aqueous humor.*

The **sclera,** or external coat, is dense and consists of connective-tissue fibres which interlace. The elastic fibres are fine and more abundant at the inner surface. The cellular elements are not numerous and many contain pigment granules. Anteriorly the sclera becomes continuous with the cornea. Posteriorly it is perforated for the passage of the nerve fibres of the optic nerve, this perforated region being known as the *lamina cribrosa.* Considerable pigmentation occurs at the margin of the cornea, at the optic-nerve entrance, and on the internal surface of the sclera; this pigmented layer is lined by a layer of flat endothelial cells known as the *lamina fusca.* The posterior portion of the external surface of the sclera is covered by a layer of endothelial cells which belong to the lymph sac known as the *capsule of Tenon.* Anteriorly the *scleral conjunctiva* is attached to the external surface of the sclera by loose connective tissue. At the junction of the sclera with the cornea—*sclero-corneal junction*—there is a venous sinus, the *canal of Schlemm.* This canal surrounds the cornea and

runs in the inner portion of the sclera. Sometimes it consists of several canals separated by connective-tissue septa.

The **cornea** is the anterior transparent, convex portion of the sclera. It consists of the following layers: (1) *anterior epithelium;* (2) the *anterior elastic membrane,* or membrane of Bowman; (3) the *substantia propria,* or ground substance; (4) the *posterior elastic membrane,* or membrane of Descemet; (5) the *posterior endothelium,* or endothelium of Descemet.

The *anterior epithelium* is of the stratified squamous type, being laid down in from six to eight layers. The basal cells are cylindrical in shape and vary in their height; the cells of the middle layers are polygonal in shape and possess spines or prickles which are short and difficult to demonstrate; the surface cells are flat.

The *anterior elastic membrane* (membrane of Bowman) is thick and homogeneous in appearance. Its thickness decreases as the sclera is approached, and it finally disappears.

The *substantia propria* consists of fine connective-tissue fibres united into bundles and held together by a cement substance. These bundles form layers or lamellæ, which in the human cornea are sixty in number. The lamellæ run parallel to the surface of the cornea, and are so arranged that the fibrils of one lamella cross those of another at an angle of about twelve degrees. The lamellæ are bound to each other by an interlamellar cement substance. The lamellæ are also held together by a series of fibres which perforate them, the *perforating* or *arcuate fibres.*

Between the lamellæ are irregular-shaped cell spaces, each containing a thin cell with many irregular processes. The cell spaces are connected by a series of fine canals, the canaliculi, which communicate with the lymph channels at the margin of the cornea. The cell spaces, when seen in sections cut perpendicular to the surface of the cornea, are fusiform in shape.

The *posterior elastic lamina* (membrane of Descemet) is thin and homogeneous in appearance. At the margin of the cornea it becomes thicker.

The *posterior endothelium* (endothelium of Descemet) is a single layer of flat hexagonal-shaped cells the nuclei of which often project slightly.

The cornea contains no blood vessels, but in pathologic conditions the vessels surrounding the margin may grow into it.

The **choroid** is made up of the following layers: (1) the *lamina suprachoroidea;* (2) the *vascular layer* (Haller's layer, layer of straight vessels); (3) the *chorio-capillaris* (capillary layer); (4) the *vitreous membrane* (membrane of Bruch, lamina vitrea). As the choroid approaches the anterior third of the eyeball it widens out, forming the *ciliary body.* From the anterior portion of the ciliary body a thin membrane, the *iris,* is given off.

The *lamina suprachoroidea* is the external layer of the choroid. It consists of several thin lamellæ of connective tissue with numerous pigmented cells. The lamellæ are covered by a single layer of endothelial cells, and the narrow spaces between the lamellæ are lymph channels, the *perichoroidal lymph spaces.*

The *vascular layer* is composed of connective tissue containing pigmented cells. It contains the large blood vessels, around which are packed many pigmented cells. On the inner surface of this layer is a narrow zone free from pigmented cells and rich in elastic fibres. This is known as the *boundary zone.* In many of the lower animals this zone consists of several layers; it is known as the *tapetum fibrosum,* and gives the peculiar shining appearance seen in the eyes of these animals.

The *chorio-capillaris* is a thin layer free from pigmented cells, and contains a network of capillary blood vessels.

The *vitreous membrane,* or membrane of Bruch, is a thin, homogeneous membrane and forms the inner layer of the choroid.

The *ciliary body* consists of the *ciliary processes* and a ring of smooth muscle, the *ciliary muscle.* It extends from the *ora serrata* (a wavy edge indicating the limit of the nervous elements of the retina) to the base of the iris.

The ciliary processes, about seventy in number, are triangular-shaped folds of the choroid, from the inner surface of which are given off secondary folds of very irregular shape. The inner surfaces of the folds are covered by the vitreous membrane, and internal to this there are two rows of low cylindrical epithelium derived from the retina, the *pars ciliaris retinæ.* The external row of these cells is pigmented, the internal row non-pigmented. The pars ciliaris retinæ continues forward to the base of the iris and

there passes into its posterior layer. The ciliary processes contain several invaginations lined with clear epithelial cells, the so-called *ciliary glands.*

The *ciliary muscle* is a circular band of smooth muscle, which appears triangular in shape in section. It lies in the outer and anterior portion of the ciliary body. It is divided into three divisions: the outer or meridional; the middle; and the inner or circular (circular muscle of Müller). The main portion of the muscle arises from the internal surface of the sclera close to the cornea, some of its fibres being attached to the ligamentum pectinatum. The fibres pass backward and are inserted in the choroid near the ora serrata. The circular division fibres have a circular course around the base of the iris.

The ciliary body is attached to the sclero-corneal junction by the *ligamentum pectinatum,* which is a continuation of the posterior elastic lamina of the cornea. At the margin of the cornea the posterior elastic lamina splits up into fibres which enclose spaces, the *spaces of Fontana.* These spaces are lined with endothelial cells and communicate with the anterior chamber. Some of the fibres of the ligamentum pectinatum pass into the substance of the iris.

The *iris* is a circular membrane behind the cornea and in front of the lens. It is to the iris that the color of the eye is due. Its centre is perforated—the *pupil*—which in man is round, while in some animals it is oblong.

The iris is composed of four layers: (1) the anterior endothelium; (2) the stroma; (3) the posterior vitreous layer; and (4) the pigmented layer.

The *anterior endothelium* is a single layer of non-pigmented cells, being a continuation of the posterior endothelium of the cornea.

The *stroma,* anteriorly, consists of a zone of reticular tissue (reticular layer) rich in cells, many of which are pigmented. The posterior portion (vascular layer) contains numerous blood vessels with thick connective-tissue sheaths. In the posterior portion of the stroma near the margin of the pupil is a layer of smooth muscle, the *sphincter muscle of the pupil.* This muscle consists of two sets of fibres: the circular, which surround the edge of the

pupil—the *sphincter of the pupil*—and a set of radial fibres, the *dilator of the pupil.*

The *posterior vitreous membrane* is the continuation of the membrane of Bruch and has the same structure.

The *pigmented layer* is composed of pigmented epithelium, being the continuation of the pigmented layers of the ciliary processes.

The **retina,** or nervous coat, consists of the following layers from without inward:

1. The layer of pigmented epithelium.
2. The rods and cones.
3. The outer nuclear layer.
4. The outer reticular or molecular layer.
5. The inner nuclear layer.
6. The inner reticular or molecular layer.
7. The layer of nerve cells.
8. The layer of nerve fibres.

These layers are not continuous throughout the entire retina, but are modified in certain regions, as the *macula lutea* (yellow spot), the *optic-nerve entrance,* the *ora serrata,* and the *pars ciliaris retinæ.*

The *pigmented epithelium* consists of a layer of hexagonal-shaped cells containing granules of pigment. The internal surfaces of these cells give off fringe-like processes which pass between the ends of the rods and cones. The distribution of the pigment varies in these cells. If the eye has been exposed to bright light just previous to its removal, the pigment is found to have collected in the processes of the cells; if, on the other hand, the eye has been kept in the dark, the pigment is found chiefly in the cell body.

The *layer of rods and cones* is composed of two kinds of neuro-epithelial elements, the *rods* being of an elongated cylindrical shape, measuring from 40 μ to 50 μ in length and 2 μ in diameter. The *cones* are shorter and thicker than the rods, measuring from 15 μ to 25 μ in length and 6 μ in diameter, and being somewhat flask-like in shape. Both the rods and cones are divided into an outer and inner segment. The former is homogeneous in structure; the latter consists of two parts, an outer, having a striated

appearance, and an inner which is granular. The outer segment of the rods is cylindrical, that of the cones short and conical. The inner segment of both bulge, that of the cones more so than that of the rods.

The total number of cones in the human retina is estimated to be about three millions; that of the rods at least three times this number. From three to four rods lie between two cones. In the *macula lutea* the rods are absent.

The *outer nuclear layer* consists of the *rod granules,* the *cone granules,* and the expanded portion of *Müller's fibres.* The *rod granules* are fibre-like structures with enlargements which may occur in any part of the fibres, each enlargement containing an oval-shaped nucleus. The outer ends of the granules are connected with the rods; the inner ends pass into the inner reticular layer and terminate in knob-like endings. The *cone granules* are broad and rest by an expansion on the outer reticular layer. An enlargement containing the nucleus lies at their outer ends where they are connected with the cones. From their inner ends fibres are given off which pass into the outer reticular layer. The rods and cones with their granules are now considered as cells, being known as the *visual cells.*

The *outer reticular or molecular layer* consists of portions of Müller's fibres, the knob-like terminations of the rod granules, the bush-like termination of the cone granules, and the dendritic process of cells of the inner nuclear layer, and has a granular or reticular appearance.

The *inner nuclear layer* consists of the nucleated portion of Müller's fibres, the *horizontal ganglion cells;* the *bipolar ganglion cells;* and the amacrine cells, so-called *spongioblasts.* The cells of this layer send dendrites into both the outer and inner reticular layers.

The *inner reticular* or *molecular layer* consists of processes of cells of the inner nuclear layer and the layer of nerve fibres and portions of Müller's fibres.

The *layer of nerve cells* consists of a single layer of cells the dendrites of which pass into the inner reticular layer and their neuraxes becoming continuous with the nerve fibre layer.

The *layer of nerve fibres* consists of non-medullated nerve fibres

of the optic nerve, the most of which are continuous with the neuraxes of the nerve cells.

Müller's fibres, or sustentacular fibres, are for the support of the retinal elements. They pass through all of the layers of the retina to the layer of rods and cones. They commence at the surface of the retina in broad basal plates, which may be forked. Their bases are united, forming the so-called internal limiting membrane. As they pass through the layers of nerve fibres and cells their surface is generally smooth. They gradually diminish in size, and in passing through the inner reticular layer give off short, transverse, hair-like processes. Upon entering the inner nuclear layer the fibres flatten somewhat and give off flat processes from their sides. This portion of the fibre contains the nucleus, which is generally situated on one side. In the outer reticular layer the fibres give off short lateral processes. In the outer nuclear layer they break up into fibrils and lamellæ, which surround the rod and cone granules. At the bases of the rods and cones the fibres terminate in end plates which blend with one another, forming the so-called external limiting membrane. The end plates give off short fibres, forming the *fibre baskets* in which the bases of the rods and cones rest.

By means of the Golgi and methylen blue methods the minute structure and relations of the various elements of the retina have been worked out by Ramôn y Cajal, whose description we shall follow.

The bases of the rods and cones are continuous with the rod granules and cone granules, which lie in the outer nuclear layer. The rod granules pass into the outer reticular layer and terminate near its outer edge in knob-like expansions. The cone granules break up into fibrils which pass into the outer molecular layer and end in arborizations.

The *inner nuclear layer* consists of: (1) *Horizontal cells* which lie along the outer edge of the layer. These cells give off dendrites which pass into the outer reticular layer; their neuraxes run horizontally, giving off collaterals which end in terminal bushes (telodendria) in the same layer. (2) *Bipolar cells,* which are of two kinds: (*a*) *rod bipolars,* (*b*) *cone bipolars.* The *rod bipolars* give off dendrites which pass into the outer reticular layer,

there intertwining with arborizations of the cone granules. Their neuraxes pass into the inner reticular layer and end in terminal bushes near the nerve cells. The *cone bipolars* give off dendrites which pass into the outer reticular layer and end around the terminations of the cone granules. Their neuraxes pass into the inner reticular layer and come in contact with the dendrites of the nerve cells. (3) The *spongioblasts* lie along the internal edge of this layer and send their processes into the inner reticular layer.

The *inner reticular* or molecular layer is composed of the arborizations of the processes of the spongioblasts, the terminal bushes of the rod bipolars, the terminal bushes of the cone bipolars, and the dendrites of the nerve cells.

The *nerve-cell layer* consists of oval-shaped nerve cells laid down in a single row. Their dendrites pass into the internal reticular layer and their neuraxes into the layer of nerve fibres. According to the manner of the termination of the dendrites, these cells are divided into three groups: *(a)* those whose dendrites pass but a short distance into the internal reticular layer; *(b)* those whose dendrites pass nearly through the internal reticular layer; and *(c)* those whose dendrites are distributed throughout the entire thickness of the inner reticular layer.

The *nerve-fibre layer* consists of two sets of fibres, the neuraxes of the nerve cells and the *centrifugal* fibres which pass into and end in the various layers of the retina.

THE OPTIC NERVE.

The optic nerve, within the orbital cavity, is surrounded by an *external sheath* (dural sheath) of connective tissue, which is a prolongation of the dura mater of the brain. Anteriorly this sheath blends with the sclera. Internal to this is the *pial sheath,* a prolongation of the pia mater. The two sheaths are separated by a fissure, the *subdural space,* which is bridged over by connective-tissue trabeculæ. The *pial sheath* is divided into two layers, the external or fibrous (arachnoid) and the internal or vascular. These two layers are separated by a narrow cleft, the *subarachnoid space,* which is also crossed by connective-tissue trabeculæ. The vascular layer of the pial sheath sends septa into

the interior of the nerve, which form elongated chambers in which the nerve fibres run.

The nerve fibres are medullated, but are without a neurilemma or sheath of Schwann. The nerve fibres are supported by neuroglia cells. As the nerve fibres reach the region of the sclera they lose their medullary sheath and become naked axis cylinders, which pass through openings in the sclera and choroid—the *lamina cribrosa*—into the interior of the eye and, radiating, become the nerve-fibre layer of the retina. At a point about 2 cm. posterior to the eyeball the central artery of the retina, accompanied by the vein, enters the optic nerve and passes to its axis. Upon reaching the interior of the eyeball the artery divides into two branches, which in turn branch and supply the retina.

THE VITREOUS BODY.

The vitreous body consists of a semi-fluid substance which contains fibres running in all directions. It is surrounded by a delicate membrane, the *hyaloid membrane,* which lies close to the retina. At the ora serrata it passes forward on the internal surface of the pars ciliaris retinæ, bridging across the recesses of the ciliary processes. In this region it is known as the *zonula ciliaris,* or *zonule of Zinn.* At the heads of the ciliary processes it splits into two layers, the anterior going to the anterior lens capsule, the posterior to the posterior capsule, forming the *suspensory ligament of the lens.* A semi-triangular space between the two layers is known as the *canal of Petit.*

The vitreous body fills the posterior chamber of the eyeball and in the normal state is transparent.

THE LENS.

The lens is a transparent, solid body, biconvex in shape. It is surrounded by a transparent, homogeneous membrane, the *lens capsule,* which is much thicker on the anterior than on the posterior surface. The internal surface of the anterior capsule is lined with a single layer of flat, polygonal-shaped cells. The body of the lens is composed of long, ribbon-shaped fibres with serrated

edges. These fibres are laid down in layers, the course of which is extremely complicated. They originate from elongated epithelial cells, the nuclei of which disappear in the adult stage. In transverse section the lens fibres appear hexagonal in shape.

THE EYELID.

The eyelid consists of three layers, the *external* or *cuticular,* the *middle,* and the *internal* or *conjunctival.*

The *cuticular layer* is covered with a thin epidermis; the derma has but few and low papillæ, which at the margin of the eyelid become much bigger. There are fine hairs with small sebaceous glands and a few sweat glands distributed through it. At the anterior margin of the eyelid there are several rows of large hairs, the *cilia,* or *eyelashes,* embedded in hair follicles. In addition to the sebaceous glands the posterior row of follicles have modified sweat glands, the *glands of Moll.*

The *middle layer* contains the *orbicularis palpebrarum muscle* and the *tarsal cartilage,* a plate of dense fibrous tissue in which are embedded the *Meibomian* or *tarsal glands,* some thirty or forty in number. These glands consist of a long tubular duct lined by stratified squamous epithelium, into which open numerous simple or branched alveoli. These alveoli are lined with stratified cuboidal epithelium. The ducts open on the margin of the eyelid posterior to the eyelashes.

The *inner or conjunctival* layer is a mucous membrane the free surface of which is covered with pseudo-stratified cylindrical epithelium (epithelium with two rows of nuclei). At the margin of the eyelid the conjunctiva becomes continuous with the skin of the eyelid. It is also continuous with the conjunctiva covering the anterior portion of the sclera (bulbar conjunctiva). The point where the conjunctiva is reflected from the surface of the eyelid on to the eyeball is the fornix conjunctivæ, or conjunctival fold.

Practical Study.

General Dissection of the Eye.—The eyes of an ox are carefully removed from the orbital cavity and all of the orbital fat and

muscle cut away from the sclera. They are then hardened in a 10 per cent. solution of formalin. After a week they are thoroughly washed in running water, when they are ready for dissection. The formalin makes the cornea slightly opaque; the vitreous humor remains as transparent as during life.

For the purpose of dissection the eyeball should be divided into lateral halves with a sharp knife, the line of the incision passing through the axis of the optic nerve.

One of the halves is placed in a dish of water, cut surface uppermost.

1. Identify the following parts: the *cornea, iris, pupil, lens, vitreous body, optic-nerve entrance, sclera, choroid, ciliary processes,* and *retina.* Note that upon looking through the transparent vitreous body the ramifications of the retinal blood vessels (reddish in color) can be seen. Also note that the lens divides the cavity of the eyeball into *two chambers*—the *anterior chamber,* or *chamber of the aqueous humor,* and the *posterior chamber,* or *cavity of the vitreous body.*

2. Make a free-hand drawing showing the above-named parts and show their relation to each other.

3. Remove the half-eye from the dish, holding it with the left hand, and with the blunt end of the forceps scoop out the *vitreous,* taking care to go well under the lens. The most of the *retina* will come away with the vitreous, leaving some of the *pigmented epithelium of the retina* adhering to the inner surface of the choroid. Observe the iridescent *membrane of Bruch,* or *vitreous membrane,* the internal layer of the choroid.

4. Remove some of the *retinal epithelium* with the blunt end of the forceps, place it on a slide in a drop of water, and examine with the *low power.*

5. Remove the *lens* with its *capsule* and *zonula ciliaris* as follows: Hold the half-eye with the left hand, steady the lens with the index finger. Pass the blunt end of the forceps down *behind* the iris and in *front* of the lens. By a gentle seesaw motion of the forceps gradually work off the *zonula ciliaris* from its attachment to the inner surface of the *ciliary processes,* passing around the entire half-eye. Place the lens in a glass of water and observe

the fringe-like zonula ciliaris passing off from the edge of the lens.

6. Remove the lens from the water. With the point of the forceps cut through the *anterior and posterior lens capsule.* Then strip off the capsule from the lens and place it in the glass of water. *Observe* that the capsule is transparent and that it retains the curvatures of the lens.

7. With the forceps strip off some of the *lens fibres.* Note that they are laid down in layers. Mount a few of the fibres in *eosin-glycerin* and examine them with the *high power.*

8. Return to the half-eye. *Observe* the form and attachment of the *iris* and the shape of the *pupil;* also the form and arrangement of the *ciliary processes.* Identify the *ora serrata.*

9. Remove the *choroid* as follows: Pass the blunt end of the forceps in *front* of the iris at its attachment to the ciliary processes, and break down the *ligamentum pectinatum,* passing around the whole of the margin of the cornea. Then pull away the choroid from the sclera. This is easy until the optic-nerve entrance is reached. Here it must be cut away, with the scissors, from its firm attachment to the *lamina cribrosa.* Place the removed choroid, convex side uppermost, in the glass of water. *Observe* its fluffy appearance, due to the external coat, the lamina supra-choroidea. Remove a bit of the fluffy layer with the forceps and place it on a slide in a drop of water, and examine with the low power. *Observe* that it is a connective-tissue membrane containing numerous pigmented connective-tissue cells.

10. Place the *choroid,* inner surface downward, over the palmar surface of the index finger of the left hand; scrape the upper surface firmly with the edge of the forceps until a series of parallel ridges appear—*layer of straight vessels, Haller's layer.* Continue the scraping until the most of the pigmented cells are removed and a thin place is obtained. Cut out the thin spot with the scissors. Stain it in *hæmatoxylin* and place it in *eosin-alcohol over night.* The next day clear and *mount* in *balsam.* Examine the thin edges with the *high power;* if the preparation has been successful the capillary vessels containing the blood cells (stained deep red)—*chorio-capillaris*—will be seen, possibly the *membrane of Bruch.* The thicker parts will show the large blood vessels of *Haller's*

layer surrounded by numerous pigmented cells, in the clear spaces between them the capillaries filled with blood.

Make a drawing of the vessels of Haller's layer, the capillaries between them, and the chorio-capillaris if it shows.

11. Examine the *internal surface* of the sclera and note the *lamina fusca.* Note the gradual passage of the translucent cornea into the dense sclera—*the sclero-corneal junction.*

Sclero-Corneal Junction, Iris, and Ciliary Body.—A human eye—that of a pig may be used—is fixed in formalin-Müller's fluid and hardened in alcohol. The eyeball is divided transversely and the anterior half embedded in celloidin. After the celloidin has been coagulated the specimen is cut so as to divide the cornea into quadrants; the lens is then carefully removed, leaving its capsule intact. Sections are made perpendicular to the surface of the cornea and are to include a portion of the sclera, the ciliary body, and iris. These sections are stained double and mounted in balsam.

Low Power.—Identify the cornea, iris, lens capsule, ciliary body, ciliary process, and ora serrata.

Make an outline drawing showing these parts.

High Power.—1. Select the cornea and identify its five layers—anterior epithelium, anterior elastic membrane, substantia propria, posterior elastic membrane, and posterior endothelium.

Make a drawing of a section through the cornea showing the minute structure of each layer.

2. Pass backward to the iris and identify the anterior endothelium, the stroma, the sphincter muscle of the pupil, and the posterior pigment layer.

Make a drawing as under the cornea.

3. Note the lens capsule.

4. Pass to the junction of the sclera and cornea. Note the difference between the structure of the sclera and the cornea. Identify the canal of Schlemm, the ligamentum pectinatum, the spaces of Fontana, the ciliary muscle, the ciliary artery. Pass inward and identify the ciliary process. Note its layers—the pigment layer, the vitreous membrane, the pars ciliaris retinæ, the zonula ciliaris, the suspensory ligament of the lens, and the canal of Petit.

Make a drawing showing the minute structure of the above parts.

5. Pass backward and note the ora serrata.

Section of the Optic Nerve and Optic-Nerve Entrance.—The posterior portion of the eyeball used for the sections of the sclero-corneal junction (see above) are utilized. The optic nerve is cut transversely at some distance from the eyeball. The entrance of the optic nerve and a portion of the coats of the eyeball are removed. All are embedded in celloidin and mounted so that transverse sections of the optic nerve and longitudinal sections of the optic-nerve entrance are made at the same time. These sections are stained **double** and mounted in **balsam.**

Low Power.—Select the transverse section of the optic nerve. Identify the dural sheath, the pial sheath, the subdural space. Note the prolongations of the pial sheath into the interior of the nerve, and the sections of the nerve fibres.

Make an outline drawing showing the above parts.

Next pass to the section of the optic-nerve entrance. Note the dural and pial sheaths, cut longitudinally, and that the latter blends with the sclera. Note the nerve fibres passing through the lamina cribrosa. Also note that after they reach the interior of the eyeball they pass to the internal surface of the retina.

Make an outline drawing showing one-half of the optic-nerve entrance.

Section of the Sclera, Choroid, and Retina.—The posterior segment of the eyeball from the human subject or pig is fixed in formalin-Müller's fluid, hardened in alcohol, and embedded in celloidin. Sections are cut perpendicular to the internal surface of the retina. These are stained **double** and mounted in **balsam.**

1. **Low Power.**—Note the relations and thickness of the sclera, choroid, and retina.

High Power.—Observe the layers of the choroid—the *lamina suprachoroidea,* the *layer of straight vessels,* or Haller's layer, the *chorio-capillaris,* the *vitreous membrane,* or membrane of Bruch.

Make a drawing of a section through the choroid showing the minute structure of these layers.

2. **Low Power.**—Observe the layers of the retina—the *pigmented epithelium,* the *layer of rods and cones,* the *outer nuclear*

layer, the *outer reticular* or *molecular layer,* the *inner nuclear layer,* the *inner reticular* or *molecular layer,* the *layer of nerve cells,* the *layer of nerve fibres.*

High Power.—Observe the appearance of each layer and make a drawing showing this appearance.

Eyelid.—The human eyelid is removed and pinned out on sheet cork, skin side downward. It is then fixed in formalin-Müller's fluid, hardened in alcohol, and embedded in celloidin. Sections are made perpendicular to its external surface. These are stained **double** and mounted in **balsam.**

Low Power.—Identify the three layers of the eyelid—the *external,* or cuticular; the *middle,* containing the *tarsal cartilage* and *orbicularis muscle;* the *Meibomian gland;* the *conjunctiva.* Pass to the margin of the eyelid and note the *eyelashes* and the *gland of Moll* (in some sections this gland will not show).

Make a drawing showing the above parts.

PART FOUR.

The Central Nervous System.

BY

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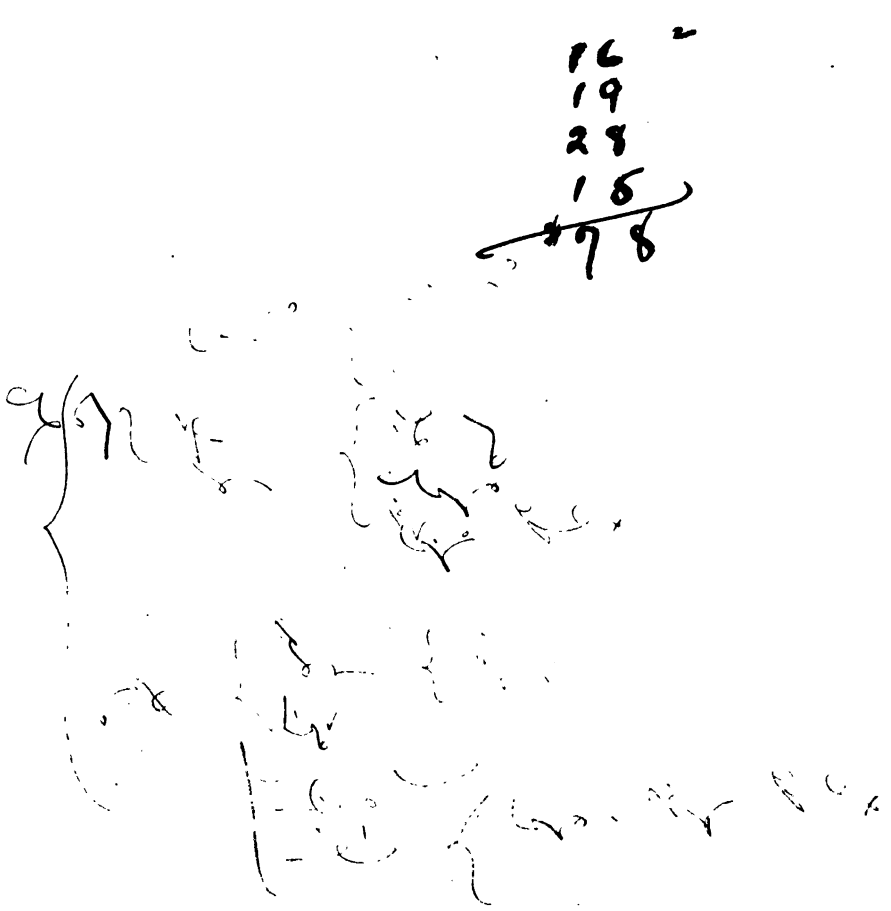

THE CENTRAL NERVOUS SYSTEM.

The nervous mechanism in man consists of two distinct though associated systems, the *cerebro-spinal nervous system* and the *sympathetic nervous system.* Each of these systems is composed of a *central portion* which is its centre of nervous activity, and a *peripheral portion* which serves to place the centre in connection with the organs which it controls. In the cerebro-spinal system the central portion is known as the central nervous system, and consists of the cerebro-spinal axis or brain and spinal cord. The peripheral portion is formed by the cranial and the spinal nerves. The central portion of the sympathetic system consists of a series of ganglia from which the sympathetic nerves take origin. These latter constitute its peripheral portion.

STRUCTURE OF NERVE TISSUE.

Reduced to its simplest terms, nerve tissue is made up primarily of *neurones.* These neurones, in their association with one another in forming the organs of the nervous system, are supported and held together by a peculiar form of connective tissue called *neuroglia. The neurone is the nerve cell with all its processes.* It is the structural unit of the nervous system considered as an organ, in the same sense that the liver cell is the structural unit of the liver. In the embryo the neurone is first distinguishable as one of the small round cells which constitute the epiblastic lining of the primitive neural canal. This embryonic nerve cell or *neuroblast* is entirely devoid of processes. Soon, however, the previously round cell becomes pyriform and from the smaller end a process begins to grow out. This process is known as the *axone* (axis-cylinder process, neuraxone, neurite). Other processes of different nature shortly appear as outgrowths of the cell body. These are known as *protoplasmic processes* or *dendrites.* Each

adult neurone thus consists of a cell body, and, passing off from this cell body, two kinds of processes, the axis-cylinder process and the dendritic processes.

The Cell Body.—Like most other cells, the nerve cells consist of a mass of protoplasm containing a nucleus. They vary in size from very small cells, such as those found in the granule layers of the cerebellum and olfactory lobe, to the large Purkinje cells of the cerebellum and the motor cells of the ventral horn of the cord, which are among the largest cells of the body. There is as much variation in shape as in size, and some of the shapes are characteristic of the region from which the cells come. Thus the cells of the spinal ganglia are spheroidal, most of the cells of the cortex cerebri pyramidal, the cells of Purkinje pyriform, the cells of the ventral horn of the cord irregularly stellate. According to the number of processes which they give off, nerve cells are often called *unipolar, bipolar,* or *multipolar.*

Our knowledge of the internal structure of the nerve cell has been greatly increased within the last few years by the use of a special technique known as the method of Nissl. When subjected to this technique nerve cells present two very different types of reaction. In certain cells only the nuclei stain, the cell body remaining perfectly clear. Such cells are found in the granule layers of the cerebellum, olfactory lobe, and retina. They are known as *caryochromes.* Other cells—and these are by far the most numerous—react, both as to their nuclei and as to their cell bodies, to the Nissl stain. These cells are known as *somatochromes.* Taking as an example of this latter type of cell one of the motor cells of the ventral horn of the cord and subjecting it to the Nissl technique, we may note the following structure:

(1) **The Nucleus.**—*(a)* A *nuclear membrane* which stains a light blue and is continuous with

(b) An *intranuclear network,* or *nucleo-reticulum,* which also takes a faint blue stain, but is easily decolorized and then restains readily with an acid dye, *e.g.*, eosin or erythrosin.

(c) A clear ground substance or *nucleoplasm,* unstained.

(d) A *nucleolus* which stains an intense blue. Instead of a single nucleolus there may be two or more nucleoli or bodies resembling nucleoli.

(2) **The Cytoplasm.**—In the cell body two distinct elements appear—a clear, unstained *ground substance,* and, scattered through this, masses of granules which take a deep blue stain and are known as *chromophilic bodies.* These bodies are granular in character; may be large or small, regular or irregular in shape; may be arranged in rows or in an irregular manner; may be close together, almost filling the cell body, or quite separated from one another. Sometimes there is a large mass of chromophilic substance at one pole of the nucleus, the so-called *"capuchon nucléaire,"* or *nuclear cap.* Another common place for accumulation of the substance is the first bifurcation of a protoplasmic process. This mass from its shape has been called the *"cone de bifurcation,"* or *division cone.* Presenting variations in different types of cells, the appearance of these bodies remains constant for a given type and has thus furnished a basis for classification.

Though the method of Nissl shows the basement substance of the cell as clear and homogeneous, or granular, the application of other methods of staining has led most investigators to ascribe to it a definite structure. This structure seems to be of the nature either of a true cytoreticulum or of delicate fibrillæ lying in the more or less homogeneous semi-fluid basement substance of the cytoplasm, and extending not only throughout the body of the cell but into its processes.

Our conception of the physiological significance of these different structural elements of the nerve cell is mainly theoretical. As has been stated, the nerve cell is the *genetic* or *birth centre* of the neurone. From the behavior of the processes when cut off from the body of the cell, it is evident that the cell body is the *trophic* or *nutritive* centre of the neurone. It seems probable that, from the standpoint of neurone activity, the cell body acts as the *functional centre* of the neurone, while the processes act mainly as means through which impulses are received and distributed. The absence of chromatic substance in the axone, its diminution during functional activity and in fatigue, taken in connection with its behavior under certain pathological conditions, favor the theory that the chromatic substance is a food element of the cell. The reticular or fibrillated part of the achromatic portion of the cell is

believed to be continuous with the fibrillæ of the axone, and is looked upon by most investigators as the true nervous mechanism of the cell. As to the relation which the appearance presented by the Nissl stained cell bears to the condition of the protoplasm of the living cell, much uncertainty still exists, some investigators believing that the Nissl bodies, as such, exist in the living cells, others that they are but precipitates due either to postmortem changes or to the action of fixatives.

Many nerve cells contain more or less brownish-yellow pigment. This pigment is not present in the cells of the new-born, but appears in increasing amounts with age. Its significance is not known.

Protoplasmic Processes, or Dendrites.—These resemble the cell body in structure, containing similar elements. The chromatic substance is in the form of elongated rods whose long axes correspond to those of the processes. The processes divide dichotomously, become rapidly smaller, and usually end (if we except the peripheral process of the cerebro-spinal ganglion cell—see below) at no great distance from the cell body. The method of Golgi shows these processes to be studded with minute projections known as *"gemmules."* Dendrites are believed to transmit impulses toward the cell body.

The Axone or Axis Cylinder Process—so-called because it usually becomes the axis cylinder of a nerve fibre—is, as a rule, single. It arises from the body of the cell or more rarely from one of the larger protoplasmic trunks. It differs from the dendrites in Nissl preparations in always originating from an area of the cell body free from chromatic substance and in being itself entirely achromatic. In Golgi preparations it is distinguished by its straighter course, uniform diameter, and smooth outline. It sends off few branches and these at approximately right angles *(collaterals)*. In certain cells the axone extends a great distance from the body of the cell. In others it branches rapidly and ends in the gray matter in the vicinity of its cell of origin. Both axone proper and collaterals usually end in terminal arborizations. They are believed to conduct impulses always away from the cell (with the possible exception of the peripheral process of the

cerebro-spinal ganglion cell—see below). Axones at their origin from the cell body and near their termination are uncovered by any sheath. An axone may pass from its cell of origin to its termination without having any sheath; such axones are confined to the gray matter. An axone may be enveloped only by a thin membrane, the *neurilemma* or sheath of Schwann. Such axones are found mainly in the sympathetic system. An axone may be surrounded by a sheath of considerable thickness known as the *medullary sheath.* Such axones are called *medullated nerve fibres* and make up the bulk of the white matter of the brain and cord. Some fibres, such as the medullated fibres of the peripheral nerves, have, in addition to the medullary sheath, a neurilemma. (For structure of a medullated nerve fibre see p. 92.)

The cell bodies of neurones are grouped mainly in the gray matter of the brain and cord, in the ganglia of the cranial, spinal, and sympathetic nerves, and in the peripheral end organs of certain of the nerves of special sense. The dendrites ramify mostly in the gray matter. The axones, on the other hand, while, as stated above, sometimes terminating in the gray matter near their cells of origin, make up the bulk of the white matter of the brain, cord, and peripheral nerves.

Neuroglia.—In addition to the fibrillar connective tissue of the ordinary type which accompanies the blood vessels as they pass inward from the pia mater, there is found in both gray and white matter a tissue unquestionably supportive in function and peculiar to the nervous system—the *neuroglia.* Unlike other connective tissue, neuroglia is of epiblastic origin, being developed from the epiblastic cells which line the embryonic neural canal.

These cells, at first morphologically identical, soon differentiate into *neuroblasts* or future nerve cells and *spongioblasts* or future neuroglia cells. In the adult two main types of neuroglia cells are found: *(a) spider cells* with thread-like, straight, unbranching processes, and *(b) mossy cells* with rough, thick, branching arms. As in the case of the nerve cell, the processes of these cells do not anastomose, but interlace, forming a dense feltwork. Spider cells occur chiefly in white matter, mossy cells in the gray matter in connection with blood vessels.

Practical Study.

Human Spinal Cord; *Transverse section; Stained by the method of Nissl.*—The cord is cut transversely into *thin* segments, which are fixed and hardened in absolute alcohol and embedded in celloidin. Thin sections are gently heated in a 1 per cent. aqueous solution of methylen blue until the fluid begins to steam. They are then washed in water and decolorized in absolute alcohol, after which they are cleared in xylol and mounted in xylol-damar. Before clearing, sections may be counterstained with a weak alcoholic solution of eosin. Mount in **balsam.** *This section is for the purpose of studying the structure of the nerve cell as shown by Nissl's method.*

Low Power.—Find an anterior horn with its group of large nerve cells stained blue. Select a cell showing *nucleus, nucleolus,* and *several processes,* and turn on the

High Power.—Note the shape of the cell body; its *dendritic processes.* Find an *axone* and note in what respects it differs from the dendrites; also that it originates from a *portion of the cell which is free from chromophilic bodies.* Note the large, round *nucleus* situated approximately in the centre of the cell: that it is surrounded by the *nuclear membrane,* stained blue, and traversed by the *intranuclear network* or *nucleoreticulum,* stained a pale blue. Within the nucleus may be seen the *nucleolus,* which takes an intense blue stain. There may be two or more nucleoli. The *ground substance* of the nucleus is seen to remain unstained or to take a very pale blue stain. Observe the shape, size, and arrangement of the *chromophilic bodies* both in the body of the cell and in the dendrites. Note that the rest of the cytoplasm, seen between the chromophilic bodies, is either unstained or stained a very pale blue.

Make a drawing of one or more cells showing the above details.

Section of the Cortex Cerebri, *stained by the Golgi silver process.*—The tissue is placed from four to six days in the following mixture:

Acid, osmic, 1 per cent. aqueous solution, 1 vol.

Potassium bichromate, 3 per cent. aqueous solution, 4 vols.

It is then transferred without washing to a 1 per cent. aqueous

solution of silver nitrate, where it remains for from twenty-four to forty-eight hours, after which it is washed in strong alcohol and rapidly embedded in celloidin. Sections are mounted in xylol-damar.

This section is for the purpose of studying the *external morphology* of the neurone and of the neuroglia cells.

Low Power.—Note the shape of the nerve cells; their coarse, rapidly branching dendrites; their long, slender, straight axones with their collaterals. Note also the two kinds of neuroglia cells, the spider cells and the mossy cells.

High Power.—Observe the finer details of structure. Can you make out any "gemmules" on the protoplasmic processes? Make drawings (1) of a neurone, (2) of a mossy cell, (3) of a spider cell.

THE CEREBRO-SPINAL AXIS.

The Spinal Cord.—The spinal cord, encased in its membranes, lies rather loosely in the vertebral canal, extending from the upper border of the first cervical vertebra or atlas above to the middle or lower border of the first lumbar vertebra below. It is cylindrical in shape and continuous above with the medulla oblongata, while terminating below in a slender cord, *the filum terminale*. At two levels of the cord, one in the cervical, the other in the lumbar region, the diameter of the cord is considerably increased. These are known respectively as the *cervical* and the *lumbar enlargements*. The posterior median *septum* and the anterior median *fissure* almost divide the cord longitudinally into two symmetrical halves. The *spinal nerve roots*, leaving the cord at intervals, serve to divide it into transverse segments, each segment extending above and below the nerve roots one-half the distance to the next adjacent roots. There are 31 of these segments, corresponding to the 31 spinal nerves; 8 cervical, 12 dorsal, 5 lumbar, 5 sacral, and 1 coccygeal. Internally the cord consists of a *central gray matter* surrounded by a peripheral zone of *white matter*. The gray matter consists mainly of nerve cells and their processes and neuroglia; the white matter of medullated nerve fibres and neuroglia. While this structure obtains for all levels of the cord, the shape of the gray matter and the relative pro-

portion of gray matter and white matter vary in different levels. The internal structure of the cord can be best studied by means of transverse sections taken at several different levels.

Practical Study.

Transverse Section of Human Spinal Cord through the Cervical Enlargement. Stained with picro-acid fuchsin (see page 94). Mount in **balsam.**

Naked Eye and Dissecting Microscope.—Note the large size of the cord—larger than at any other level—and the shape of the cord, somewhat flattened dorso-ventrally; the *anterior median fissure,* quite broad and shallow, into which the pia mater extends; the *posterior median septum,* deeper and narrow, over which the pia mater passes, merely sending down into it some strands of connective tissue. Observe the thin membrane investing the cord, the *pia mater spinalis.* The gray matter is seen in the central portion of the cord, stained red and arranged somewhat in the form of the letter H. Surrounding the gray matter is the white matter, stained a reddish yellow. Posteriorly the gray matter extends almost to the surface of the cord—*posterior horns* or *cornua.* The *anterior horns,* on the other hand, are shorter and more blunt, and separated from the surface of the cord by a considerable layer of white matter. Laterally the gray matter extends out somewhat into the white matter, and this is sometimes spoken of as the *lateral horn,* or, because of the interlacing of its fibres with fibres passing longitudinally, as the *processus reticularis.* Note that the posterior horn divides the white matter into two parts, one lying between the horn and the posterior median septum, known as the *posterior column;* the other comprising the remainder of the white matter, the *antero-lateral column.* This latter is again partially divided by the anterior horn into a *lateral column* and an *anterior column.*

Low Power.—*Gray matter.* In the cross-portion of the H note the *central canal,* usually obliterated in the adult and represented only by a group of epithelial cells. The canal divides the gray matter connecting the two sides of the cord into an *anterior gray commissure* and a *posterior gray commissure.* Around the cen-

tral canal is a light granular area composed of neuroglia and known as the *substantia gelatinosa centralis.* The posterior horn is divided into an expanded *head* or *caput,* in which is an area similar to that surrounding the central canal, the *substantia gelatinosa of Rolando,* and a narrow *neck* or *cervix.* Note the structure of the processus reticularis extending out into the white matter between the anterior and posterior horns; the groups of large nerve cells in the anterior horns; and the fibres which pass out from the anterior horns to the surface of the cord—the *anterior nerve roots.* In the white matter note the connective-tissue trabeculæ which extend in from the pia mater, an especially well-marked one dividing the posterior column into a median portion next the posterior median septum—the *Column of Goll*—and a lateral portion next the posterior horn—the *Column of Burdach.* If the section has been cut through a posterior nerve root, note the *posterior root fibres* entering the white matter of the cord just to the inner side of the posterior horn.

Drawings.—Especial care should be taken in all drawings of the cord to represent accurately the relative size of the cord at the different levels and the shape and size of the gray matter.

Naked Eye and Dissecting Microscope.—Make a large outline drawing of the cord showing the points which can be so observed. Then add details above described with the *low power.*

High Power.—*Gray matter.* Note the structure and make a drawing of one or two of the large nerve cells found in the anterior horns, showing nucleus, nucleolus, granular cell protoplasm, and dendrites.

White matter. Draw several nerve fibres as seen in transverse section, showing the *axis cylinder* stained red, the *medullary sheath* stained yellow. Note the variation in size of the fibres in different parts of the white matter.

Transverse Section of the Human Spinal Cord through the Mid-dorsal Region. Stained by Weigert's method.

In Weigert's method the tissue is hardened in Müller's fluid, after which it is passed through alcohols of increasing strength. It is then embedded in celloidin and transverse sections made. These are soaked for twenty-four hours in a saturated aqueous solution of neutral cupric acetate diluted with an equal volume of

water. They are then washed thoroughly in water and placed in the following staining fluid: Hæmatoxylin crystals, 1 gm.; alcohol, 95 per cent., 10 c.c.; saturated aqueous solution of lithium carbonate, 1 c.c.; water, 90 c.c., in which they remain for from two to twenty-four hours. They are then thoroughly washed in water and decolorized in the following fluid: Potassium ferricyanid, 2.5 gms.; sodium biborate, 2 gms.; water, 300 c.c. They remain in this fluid until the gray matter becomes of a distinct yellow color, the white matter remaining a dark blue or black, after which they are washed in water, dehydrated in alcohol, cleared in oil of origanum, and *mounted in balsam*. The *medullary sheaths* alone are stained by this method and appear blue or black.

Dissecting Microscope and Low Power.—Note first the same general features as described under the cervical section. Then note the following differences: The shape and size of the section; that it is smaller and more nearly round; that while the reduction in size affects both the gray and the white matter, it is the former which is most markedly decreased. Both anterior and posterior horns are more slender and there is but little lateral expansion of the anterior horn; the processus reticularis has disappeared, although a short lateral horn tapers off into the white matter. The small anterior horns contain but few cells. At the inner side and base of the posterior horn some cells of *Clark's column or nucleus* (see below) may be present. This column is not as well developed at this level as it is in the lower dorsal and upper lumbar regions. The connective-tissue septum between the columns of Goll and Burdach is not as well marked as in the cervical region.

Naked Eye and Dissecting Microscope.—Make a drawing showing the general topography of the cord at this level.

Low Power.—Fill in all details mentioned above.

High Power.—Make a drawing of several sections of the nerve fibres. What element of the nerve tissue is stained by Weigert's method?

Transverse Section of the Human Spinal Cord at the Level of the First Lumbar Segment. Stained by Weigert's method. Mount in **balsam.**

Dissecting Microscope and Low Power.—Note the general

features already described. The size and shape of the cord are about the same as in the last section studied. The amount of white matter is somewhat less; the gray matter is increased in amount, both anterior and posterior horns being less slender. Note a group of cells already mentioned which lies in the gray matter at the inner side and base of the posterior horn. It is known as the *nucleus dorsalis, Stilling's nucleus,* or *Clark's column.* As a column of cells it extends uninterruptedly from the seventh cervical to the third lumbar segment. Isolated portions of the nucleus are also found in the cervical and in the sacral regions. The nucleus is composed of rather large cells which are usually described as being either bipolar or multipolar. Fibres are seen to enter Clark's column from the posterior columns, forming a delicate interlacement around the cells.

Make a drawing showing the general topography of the cord at this level, then fill in details with the low power. Draw carefully *Clark's column,* showing its cells and the interlacing fibres which are seen entering from the posterior columns.

Transverse Section of the Human Spinal Cord through the Lumbar Enlargement. Stained by Weigert's method. Mount in **balsam.**

Dissecting Microscope and Low Power.—Note the size of the cord—larger than in any other region except the cervical enlargement. This size is due to an increase in the gray matter, the amount of white matter remaining about the same as in the preceding section. Both anterior and posterior horns are large and thick, and the caput of the latter shows a large substantia gelatinosa of Rolando. In the anterior horn the groups of cells are large and numerous. The gray commissure is much broader than at the other levels of the cord. There is no processus reticularis, and no connective-tissue separation of the posterior columns into the tracts of Goll and of Burdach.

Make a drawing showing the general topography of the cord at this level, and fill in details with the low power.

Transverse Section of the Human Spinal Cord in the Sacral Region. Stained by Weigert's method. Mount in **balsam.**

Dissecting Microscope and Low Power.—Note the marked

diminution in the size of the cord. The gray matter is smaller than in the lumbar region, but still occupies the bulk of the cord, both anterior and posterior horns being thick, while the white matter is reduced to a narrow peripheral rim.

Make a drawing showing the general topography of the cord.

ORIGIN OF THE FIBRES WHICH MAKE UP THE WHITE MATTER OF THE CORD.

It has already been observed that the white matter of the cord is composed mainly of medullated nerve fibres, most of which run in a longitudinal direction. From our study of the neurone it follows that each of these fibres must be the axone of some nerve cell. These cells, the axones of which form the white matter of the cord, are situated as follows:

- A. Cells outside the spinal cord. (*Extrinsic cells.*)
  - (1) Cells outside the Central Nervous System (spinal ganglion cells).
  - (2) Cells in other parts of the Central Nervous System (*e.g.*, in the brain).
- B. Cells situated in the gray matter of the cord. (*Intrinsic cells.*)
  - (1) Root cells, such as those of the anterior horn, whose axones form the ventral root.
  - (2) Column cells, whose axones enter into formation of the fibre columns of the cord.
  - (3) Cells of Golgi, type II, the axones of which ramify in the gray matter. (These cells do not give rise to fibres of the white matter, but are conveniently mentioned here among the other cord cells.)

(1) The Spinal Ganglion Cell and the Origin of the Posterior Columns.—The fibres of these columns consist mainly of *ascending* and *descending* branches of the fibres, which enter the cord as the *posterior nerve roots.* Following these fibres outward, they are seen to originate in the cells of the *spinal ganglia.* In very early embryonic life the group of cells which later becomes a spinal ganglion is represented by a few *ectoblastic cells* which lie between the closing medullary plate and the external layer of the ectoblast. These cells become separated off from the medullary canal by the mesoblast. At first round, these cells which have thus migrated from the central nervous system soon become spindle-shaped, and from each end of the spindle a process grows out: one, directed toward the surface of the body, joins the axones

of the cells of the anterior horn to make up the mixed spinal nerve; the other, directed centrally, enters the cord as one of the fibres of the posterior root. In its embryonic condition the spinal ganglion cell is thus seen to be bipolar. This bipolarity remains throughout life in certain of the lower animals, as, for example, in certain fishes. In man, however, it does not continue beyond embryonic existence. During its development the two processes of the bipolar cell approach each other and in the adult are connected with the cell body by a single process. The adult spinal ganglion cell is thus apparently a unipolar cell, its single process dividing and sending one arm toward the periphery, the other toward the spinal cord. An exception to this unipolarity of the spinal ganglion cell is found in the spinal ganglion of the cochlear nerve and the ganglion of the vestibular nerve, where in man, and in mammals generally, the bipolar condition remains throughout life.

The peripheral processes of the spinal ganglia cells make up the *sensory* or *afferent* portions of the spinal nerves. The modes of termination of these peripheral processes are extremely varied and complicated. These peripheral terminations are always free, in the sense that, while possibly sometimes penetrating cells, they probably never become directly continuous with their protoplasm.

In the skin, and in those mucous membranes which are covered with squamous epithelium, the nerve fibres lose their medullary sheaths in the subepithelial tissue, and, penetrating the epithelial layer, split up into minute fibrils which pass in between the cells and terminate there, often in little knob-like swellings. In addition to such comparatively simple nerve endings, there are also found in the skin and mucous membranes, especially where sensation is most acute, much more elaborate terminations. Among these may be mentioned Merkel's tastzellen, or touch cells, the tactile corpuscles of Meissner, and the Pacinian bodies. In tendons and in muscle, sensory nerve fibres, after losing their medullary sheaths, divide into minute fibrils which are often studded with irregular expansions. Such a termination from a single nerve fibre is in many cases extremely elaborate. In gland tissue nerve fibres usually end as fine fibrils which pass to the epithelial cells.

It is important to bear constantly in mind the fact that these nerve terminals, however complicated, are in no sense nerve centres like the ganglion cells, but merely more or less elaborate end arborizations for the purpose of receiving impulses.

Because of the fact that it transmits the impulse toward its cell of origin, as well as because of certain other facts, Van Gehuchten considers this peripheral process of the spinal ganglion cell of the nature of a protoplasmic process.

The centrally directed arm of the spinal ganglion cell, which, according to Van Gehuchten, represents the true axone, enters the spinal cord as one of the fibres of the posterior root, the entire bundle of posterior root fibres of a single spinal nerve consisting of all the central axones of the corresponding spinal ganglion. Having entered the cord, the fibre divides in the posterior columns into an *ascending arm* and a *descending arm,* and these ascending and descending arms of the central processes of the cells of the spinal ganglion constitute the great majority of the fibres of the posterior columns. The descending arm is usually short, sends off branches known as collaterals into the gray matter of the cord, and itself terminates there at no great distance below its point of entrance into the cord. The ascending arm may behave in a similar manner, passing up the cord but a short distance, where, after sending collaterals into the gray matter, it also terminates in the gray matter of the cord. Instead of being short it may be of considerable length, passing some distance up the cord before finally terminating in the gray matter. It may, as one of the long fibres of the posterior columns, continue into the medulla to end in one of the nuclei of these columns. Just after entering the cord some of the most external of the fibres of the posterior root turn sharply outward and, after bifurcating, ascend and descend in a tract of fine fibres which lies between the tip of the posterior horn and the surface of the cord, extending slightly over into the posterior and into the lateral columns, and known as the *tract* or *marginal zone of Lissauer*. These fibres are short and with their collaterals terminate in the gray matter of the posterior horn. The rest of the fibres bifurcate and send their processes up and down in the external part of the *posterior column,* or *the column of Burdach*. Each successive dorsal root sends its fibres into the cord, to the

outer side of those which have entered in the next root below. Thus the fibres from the lower roots are gradually thrust inward toward the median line as they ascend the cord, finally coming to lie in the inner part of the posterior column, or the column of Goll. The most median fibres, therefore, of the column of Goll are the longest fibres of the posterior columns, having come from the lower spinal ganglia, while the column of Burdach is made up of short and of medium-length fibres and of long fibres which higher up pass over into the column of Goll. These neurones just described, whose cell bodies lie in the spinal (and cranial) ganglia, whose peripheral arms with their end organs furnish the afferent receptive apparatus, and whose central arms terminate in the gray matter of the cord and medulla, constitute the peripheral sensory or peripheral afferent neurone system.

Practical Study.

Transverse Section through a Chick of Six Days' Incubation. Golgi silver process.

Low Power.—Locate the *spinal cord.* Note the outlines of the gray matter and the white matter. Observe the *spinal ganglia* lying one on either side of the cord. One of the ganglia will probably show one or more bipolar cells, sending one process toward the periphery, the other toward the spinal cord. Note that the peripheral process is joined, beyond the ganglion, by fibres which come from the ventral region of the cord (fibres of the anterior root). In some specimens the latter can be traced to their origin in the cells of the anterior horn. The union of the peripheral processes of the spinal ganglion cells and the anterior horn fibres is seen to make up the mixed spinal nerve. Observe the central processes of the spinal ganglion cells entering the dorsal column of the cord and bifurcating. As these branches pass up and down the cord, only a short portion of each can be seen in a transverse section. Note the fibres (collaterals) passing from the white matter into the gray matter. Note in some of the sections a little round mass just below and to the side of the spinal ganglion, in which some nerve cells may be seen and some fibres passing into or out of it. This represents the beginnings of the sym-

pathetic system with its chain of ganglia. Note the relation which this bears to the spinal cord and spinal ganglia.

Make a drawing showing the spinal cord with its gray matter and white matter. Represent accurately the position and size of the spinal ganglion, and draw in detail the cells and the directions of the various fibres as described above, including the sympathetic ganglion if it is present in your section.

Longitudinal Section of a Chick of Six Days' Incubation. Golgi silver process.

The plane of section is not the same for all the specimens. Some cross the cord in the anterior or posterior region, thus showing two columns of gray matter corresponding to the anterior or the posterior horns, and three columns of white matter, one between and one on either side of the gray columns. Some sections pass through only one anterior or posterior horn, showing one central gray column and a white column on either side. Still other sections pass in a dorso-ventral plane, cutting through the anterior and posterior horns of the same side, showing a large central gray column and one small white column on either side.

Low Power.—Locate the *gray matter* and the *white matter,* and identify the plane of section. Trace the fibres entering the white matter and there dividing into ascending and descending arms, which are seen running up and down in the white columns. Note that from these longitudinal fibres branches (collaterals) pass into the gray matter, where they terminate. The origin of these fibres from the cells of the spinal ganglia has been noted in the previous section.

Make a drawing with the low power showing outlines of gray matter and of white matter and the fibres as noted above.

(2) Root Cells; Motor Cells of the Anterior Horn.—These are large *multipolar* cells found at all levels of the cord and having analogues in the motor nuclei of the cranial nerves. They are most numerous in the cervical and lumbar enlargements. In cross-sections of the cord, especially through the enlargements, a more or less definite grouping of these cells is evident. In longitudinal sections these groups are seen to extend for varying distances up and down the cord, forming cell columns or nuclei, each one of which corresponds to the innervation of a particular muscle.

Two of these cell columns are quite constant throughout the entire length of the cord. They are known, from the positions which they occupy, as the columna medialis and the columna intermediolateralis and are related to the muscles of the trunk. At certain levels these columns may be divided into secondary columns. In the cervical and lumbar enlargements other groups of nerve cells appear which are concerned in the innervation of the muscles of the extremities. They are known respectively as the cell column of the upper extremity (columna extremitatis superioris) and the cell column of the lower extremity (columna extremitatis inferioris). The dendrites of these cells ramify in the gray matter, where they intermingle with the terminal ramifications and collaterals of sensory fibres and of fibres of the direct and crossed pyramidal tracts. Their axones pass out of the ventral horn, across the fibres of the antero-lateral ground bundle, and leave the cord as the anterior, motor, or efferent roots of the spinal nerves. The fibres of this root pass by the spinal ganglion without entering it, and beyond join the fibres from the ganglion to form the mixed spinal nerve. On their way to the muscles the motor axones may bifurcate several times, thus allowing one neurone to innervate more than one muscle fibre. In the perimysium the nerve fibres undergo further branching, after which the fibrils pass to the individual muscle fibres, where they terminate in motor end plates. These neurones constitute the *peripheral motor* or *efferent neurone system.*

(3) **Column Cells.**—These lie in the gray matter of the cord and send their axones into the white matter, where they form columns of nerve fibres. Some of these cells send their axones into the white matter of the same side of the cord. These are known as *tautomeric cells.* Others send their axones to the white matter of the opposite side of the cord—*heteromeric cells.* In still others the axones divide, one branch going to the white matter of the same side, the other through the anterior commissure to the white matter of the opposite side—*hecateromeric cells.* The most important of these cells may be classified according to the fibre columns which their axones form, as

(a) Clark's column (nucleus of Stilling; nucleus dorsalis). This has already been seen in cross-section as a well-defined group

of cells just to the inner side of the root of the posterior horn. As a column of cells it extends from the seventh cervical to the third lumbar segments and is represented by isolated groups of cells in the upper cervical and sacral regions. The nucleus is composed of medium-sized bipolar or multipolar cells. These cells send their axones across the gray matter and white matter of the same side of the cord (tautomeric cells) to near its dorso-lateral surface. Here these axones turn upward to form a column of fibres which will be described later as the direct cerebellar tract. As already noted, some fibres of the posterior root (central processes of spinal ganglia cells) end, either by their terminal fibrils or by means of collaterals, in among the cells of this nucleus. The neurones of Clark's column, therefore, constitute a second neurone system in the sensory conduction path.

(b) Cells whose axones form the column of Gowers. These cells are scattered throughout the gray matter of the cord, apparently without distinct grouping. They send their axones across the intervening gray matter and white columns to the periphery of the ventro-lateral region of the cord, where they turn upward, forming the column of fibres to be described later as the tract of Gowers or the ascending antero-lateral tract. Some of these cells are situated in the gray matter of the cord on the same side as the tract which their axones enter (tautomeric cells); others are situated in the gray matter of the opposite side (heteromeric cells), their axones passing through the anterior commissure.

(c) Cells whose axones form the short-fibre tracts of the cord (fundamental columns or ground bundles). Like the preceding, these cells are distributed through the gray matter without arrangement into groups. Their axones pass into that part of the white matter which lies along the edge of the gray matter. Here they turn upward or downward, or split and send one arm up, the other down. These axones are comparatively short, send collaterals into the gray matter, and themselves terminate there at varying distances from their cells of origin. Some of these cells send their axones into the fundamental columns of the same side (tautomeric cells); others send their axones into the fundamental columns of the opposite side (heteromeric cells); in a few others

the axone divides, sending one branch to the fundamental columns of the same side, the other to the fundamental columns of the opposite side (hecateromeric cells).

(4) Cells of Golgi type II.—The axones of these cells do not leave the gray matter, but divide rapidly and terminate in the gray matter in the vicinity of the cells from which they originate.

(5) Cells from Other Parts of the Central Nervous System which Contribute Axones to the White Matter of the Cord.—The most important are the cells of the motor area of the cortex cerebri. The axones of these cells pass down the spinal cord, where they form the tracts which we shall study later and which are known as the pyramidal tracts.

Practical Study.

Transverse Section through a Chick of Six Days' Incubation. Golgi stain.

This section is for study of the *intrinsic cells of the cord.* Not all sections contain all the types of cells above described. Some contain *tautomeres* (cells whose axones pass to the white matter of the same side of the cord); others contain *heteromeres* (cells whose axones pass to the opposite side of the cord); a very few sections contain *hecateromeres* (cells the axones of which bifurcate, one arm passing to the white matter of the same side of the cord, the other to the white matter of the opposite side). Note that it is the course of the *axone alone* which determines the character of the neurone.

Low Power.—Make a drawing showing the outlines of the *gray matter* and *white matter.* Draw the type or types of cells which the section shows. Then exchange sections with your neighbors and draw any other types of cells which you may find.

Transverse Section through a Chick of Six Days' Incubation. Golgi silver process.

Low Power.—Locate the *spinal cord* and note the outlines of the gray matter. Note that the gray matter is bordered by a rim of short-cut fibres which pass from white matter into gray matter and from gray into white. These fibres fall into two classes: (1) Those situated along the inner margin of the posterior horns.

These are mainly collateral branches from ascending and descending arms of spinal ganglion cells. (2) Those passing from other parts of the gray matter into the adjacent white matter. Some of these fibres represent the axones of the *intrinsic cells* of the cord, as seen in the specimen just studied. These axones, entering the white matter, turn up or down, or, bifurcating, send one branch up, the other down, in that part of the white matter which lies adjacent to the gray matter. They thus form the *fundamental columns* or *ground bundles.* As these fibres send collaterals into the gray matter and themselves terminate there, many of the fibres entering the gray matter are collaterals and terminals of these fibres. In some of the sections the entering fibres of the posterior root (central processes of spinal ganglion cells) can be seen.

Make an outline drawing with the low power showing the gray matter and the white matter of the cord. Draw the rim of fibres surrounding the gray matter and the collaterals passing into the gray matter. If the entering fibres of the posterior root show, indicate them in the drawing.

FIBRE TRACTS OF THE CORD.

The fact that the cell bodies of neurones are grouped in the gray matter of the brain and spinal cord, in the ganglia, and in the peripheral end organs of certain of the nerves of special sense, has already been referred to. This grouping of neurones is for definite physiological purposes, their cell bodies being grouped together to form *centres* or *nuclei,* their axones following certain definite paths which are known as *fibre tracts* or *fibre systems.* If the cell bodies and dendrites be included with the axones, the whole is known as a neurone system; while if several neurone systems are concerned in the transmission of a particular set of impulses, the whole is referred to as a *conduction path.* For example, that system of neurones whose cell bodies are situated in the anterior horns and whose axones constitute the motor part of the spinal nerves is known as the spino-peripheral neurone system. If we include with this that system of neurones the cell bodies of which are located in the motor cortex and the axones of which

terminate around the anterior horn cells of the cord, the whole constitutes the motor cortico-spino-peripheral conduction path.

A nucleus which contains the cell bodies of a system of neurones is known as the *nucleus* of *origin* of that system. Thus the already referred to groups of cells in the anterior horns are the nuclei of origin for the spino-peripheral neurone system. A nucleus in which terminate the axones of a system of neurones is known as the *terminal nucleus* of that system. Thus the nucleus dorsalis, or Clark's column, serves as a terminal nucleus for some of the axones of the peripheral sensory neurone system (see page 219). In most cases a nucleus is the terminal nucleus for the axones of one neurone system and at the same time the nucleus of origin for the axones of another neurone system. Thus, in the case above cited, the nucleus dorsalis, while serving as the nucleus of termination for some of the fibres of the peripheral sensory neurone system, also serves as the nucleus of origin for a second neurone system the axones of which pass upward to higher centres.

The fibre tracts of the cord are not separated from one another by connective tissue, nor do the fibres of one tract differ in appearance from the fibres of other tracts, so that it is impossible to morphologically differentiate or to mechanically trace the different fibre systems of the cord. Certain methods of investigation, however, have enabled us to determine most of these tracts and the paths which their fibres follow. Among the most important of these may be mentioned the method of embryology and the method of pathology. The embryological method is based upon the fact that the fibres of physiologically distinct systems acquire their medullary sheaths at different periods of embryonic development. Thus, by examining cords from embryos of different ages, it is possible to distinguish the different tracts by the extent of the myelinization of their fibres. The pathological method is based upon the fact that when an axone is cut off from its cell of origin it dies and its place is taken by new connective tissue. Thus, if in any way a tract of fibres is interrupted, all of the axones of cells which are situated on the other side of the lesion atrophy and can be traced among the normal fibres. Advantage is taken of this latter method for the purpose of experimental research in animals.

ASCENDING FIBRE TRACTS OF THE CORD.

I. The Posterior Columns.—The origin of these tracts—central axones of the cells of the spinal ganglia—has been already described. Each posterior column may be subdivided into three columns: (1) the *column of Goll,* or *funiculus gracilis;* (2) the *column of Burdach,* or *funiculus cuneatus;* and (3) the *column* or *marginal zone of Lissauer.*

(a) The column of Goll (funiculus gracilis) is the median part of the posterior column, being bounded by the posterior median septum. It is largest and most distinct in the cervical region, where it is separated from the column of Burdach by a connective-tissue extension of the pia mater. It decreases in size as we pass down the cord, until in the lower lumbar region it is represented only by a small area next the median septum. The fibres of the column of Goll are, as already described, axones from cells of the lower spinal ganglia. These axones in their ascent have been pushed toward the median line by the entrance on their outer side of fibres of successive nerve roots. Most of these fibres terminate in the *nucleus of the column of Goll* (nucleus funiculi gracilis) in the medulla (see below). Some fibres probably pass this nucleus and are continued through the restiform body to the cerebellum. The nucleus gracilis—which we shall see in sections of the medulla—serves as the terminal nucleus for most of the axones of the column of Goll.

(b) The column of Burdach occupies the outer portion of the posterior column. It consists of short fibres, fibres of medium length, and of long fibres which ultimately pass over into the column of Goll. Axones of the cells of the upper spinal ganglia pass to the *nucleus funiculi cuneati*—which we shall see later in sections of the medulla—in the same way that axones from the cells of the lower spinal ganglia pass to the *nucleus funiculi gracilis* through the column of Goll. The nucleus funiculi cuneati serves as the terminal nucleus for most of the fibres of the column of Burdach. Other fibres probably pass the nucleus, as do some of the fibres of the column of Goll, to enter the restiform body and terminate in the cerebellum.

About the middle of the column of Burdach is a small bundle of

fibres which, from their behavior after injury to the cord, appear to be descending fibres. They are sometimes designated the *"comma" tract of Schultze.* This tract extends throughout the thoracic cord. Investigators differ as to the origin of its fibres, some believing that they represent descending branches of spinal ganglia cells, others that they are descending axones from cells in the gray matter of the cord.

(c) Column of Lissauer is a column of fine short fibres which lies between the tip of the posterior horn and the surface of the cord. It may extend over somewhat into the posterior and into the lateral columns. The origin of its fibres has been described (page 210). Its fibres end in the gray matter of the posterior horn.

From the posterior columns *terminal axones* and *collaterals* are constantly passing into the gray matter to end in arborizations among the cells. The gray matter of the cord thus serves as an extended nucleus of termination for the peripheral sensory system of neurones. These terminals and collaterals are distributed mainly as follows:

(a) To the dorsal and middle region of the gray matter.

(b) To the nucleus dorsalis, or column of Clark.

(c) Through the posterior commissure to the gray matter of the opposite side.

(d) To the gray matter of the ventral horns, where they end around the motor cells.

II. The Direct Cerebellar Tract (Dorso-lateral Ascending Tract, Dorso-lateral Spino-cerebellar Fasciculus, Tract of Flechsig).—This tract lies on the periphery of the cord, extending from the posterior horn to about the mid-lateral region. It is bounded externally by the surface of the cord, internally by the crossed pyramidal tract. The origin of its fibres in the cells of Clark's column has already been described. This tract first appears in the upper lumbar cord and increases in size until the upper limit of Clark's column has been reached. In the medulla these fibres pass into the corpus restiforme and so into the cerebellum through its inferior peduncle. Here they enter the gray matter, lose their medullary sheaths, and end in ramifications around the nerve cells. Some of the fibres end in the adjacent half of the vermis, others

pass through the commissure to the opposite half, still others end in the cerebellar nuclei.

III. Gowers's Tract (Antero-lateral Ascending Tract, Fasciculus Ventro-lateralis Superficialis).—This tract lies upon the surface of the cord, extending from the anterior limit of the direct cerebellar tract to the exit of the ventral root fibres. It is formed by axones of neurones whose cell bodies are located in the central part of the gray matter of the cord. Some of the fibres come from cells on the same side of the cord, others through the anterior commissure from cells on the opposite side. The tract makes its first appearance in the upper lumbar cord and increases in size as it passes upward. In lesions affecting the lower part of this tract the consequent ascending degeneration can be traced only to the middle or upper cervical region, while in any lesion of the cord causing degeneration of this tract the degenerated fibres decrease in number as the distance from the lesion increases. The explanation of this would seem to be that many of the fibres of this tract are *spinal association fibres,* and, after running a shorter or longer course up the cord, turn into the gray matter, where they terminate. The tract is not, however, a purely spinal tract, for a considerable number of its fibres continue upward to higher centres. The exact paths which these fibres take after leaving the cord, and their terminations, are still not positively determined. They have been variously described as ending in the cerebellum, in the corpora quadrigemina, in the thalamus, in the substantia nigra, and in the nucleus lentiformis. It seems probable that these varying results of investigation are due to the fact that the tract of Gowers does not represent one physiologically distinct system, but is composed of fibres having many different functions and destinations.

Practical Study.

Transverse Section of the Human Spinal Cord—cervical region. Stained by Weigert's method.

This section is from a human cord in which compression from fractured vertebræ has produced a complete transverse lesion of the cord several segments below the level at which the sections were taken.

Note the areas—stained brown or black—of normal nerve fibres, and that these areas correspond in general to the location of the *direct* and the *crossed pyramidal tracts* and the *fundamental columns* (see below). There is also a layer of fibres along the medial margin of each posterior horn. Note the areas—unstained—of degeneration, and that these correspond to the location of the already described *ascending tracts, i.e.,* the posterior columns, the direct cerebellar tracts, and the tracts of Gowers. It will be remembered that the fibres of these tracts are axones of cells which are situated below the lesion. The posterior column fibres are the ascending branches of the axones of spinal ganglion cells. It will be observed that the outer part of these columns along the medial aspect of the posterior horns contains normal fibres. These represent ascending branches of spinal ganglion cells from ganglia situated above the lesion and below the level from which the section was taken. As their connections with their cell bodies are still unbroken, they remain undegenerated. In a section taken between the lesion and the first ganglion above, all fibres would be degenerated (comma tract excepted if present). The farther the section above the injury the greater the number of posterior root fibres from ganglia above the seat of the lesion, and therefore the greater the number of normal fibres in the outer part of the posterior columns.

The direct cerebellar tracts, being composed of ascending axones of Clark's column cells, all of which are below the point of lesion, show complete degeneration of these fibres. Any normal fibres found in these tracts must be axones of other neurone systems, probably of the descending cerebellar tract.

The tracts of Gowers also show quite complete degeneration. Any normal fibres occurring within the areas of these tracts must be considered as ascending cerebellar axones from cells in the gray matter between the level of the lesion and the level of the section, or descending axones.

It is possible in this section to determine the areas occupied by the tracts of Goll and Burdach, the direct cerebellar tract, and the tract of Gowers; also the external limit of the crossed pyramidal tract, that boundary being made by the inner margin of the direct cerebellar.

Make a drawing showing the general topography of the gray matter and the white matter, and indicate the location and extent of the degenerations.

DESCENDING FIBRE TRACTS OF THE SPINAL CORD.

I. The Pyramidal Tracts.—(1) *The Crossed Pyramidal Tract.* This is a large, irregularly triangular tract of fibres lying in the dorsal part of the lateral column. It extends to the lowermost part of the cord. In the cervical and dorsal regions it is separated from the surface of the cord by the direct cerebellar tract. In the lumbar region the latter tract is no longer present and the crossed pyramidal comes to the surface.

(2) *The Direct Pyramidal Tract, or Tract of Türck*, occupies a small oval area adjacent to the anterior median fissure. It decreases in size as the lower levels of the cord are reached, to disappear entirely in the middle or lower dorsal region.

The pyramidal tracts vary greatly in size in different individuals and are apt to be asymmetrical, this being due to the lack of uniformity as to the number of fibres which cross over in the pyramidal decussation (see below).

These two tracts are the main motor or efferent fibre systems of the cord. Their location in the cord has been already described. The cell bodies of the neurones whose axones constitute these systems are situated in the *cerebral cortex* near the *fissure of Rolando*. Their axones converge and pass downward through the internal capsule, crura cerebri, pons, and medulla, sending off fibres to the motor nuclei of the cranial nerves. In the medulla the tracts come to the surface as the *anterior pyramids*. At the junction of medulla and cord occurs what is known as the *pyramidal decussation*. Here most of the fibres of each tract cross to the opposite lateral region of the cord and continue downward as the *crossed pyramidal tracts*. The minority of the fibres, instead of decussating, remain on the same side to pass down the cord along the anterior median fissure as the *direct pyramidal tracts*. As these tracts descend they decrease in size from loss of fibres which continuously leave them to terminate, those of the crossed tract in the ventral gray matter of the same side of the cord, those

of the direct tract, after passing through the anterior commissure, in the gray matter of the opposite side. These tracts are *crossed tracts,* as all of their fibres, either in the pyramidal decussation or in the anterior commissure at different levels, cross to terminate in the gray matter of the cord on the side opposite to the side of the cortex in which their cell bodies are situated. The tracts are apt to differ in size on the two sides of the cord, owing to the fact that the proportion of fibres which decussate is not constant. In the cord the fibres terminate in arborizations around the motor cells of the anterior horns, thus constituting the upper or cortico-spinal motor neurone system. It will be remembered that the neurones whose cell bodies are situated in the anterior horn constitute the spino-peripheral motor neurone system. These two neurone systems form the cortico-spino-peripheral motor conduction path.

II. The Antero-lateral Descending Cerebellar Tract (Anterior Marginal Bundle of Loewenthal) consists of descending axones of neurones whose cell bodies are situated in the cerebellum, either in the cerebellar cortex or in some of the cerebellar nuclei. Some of the fibres probably come from cells in the inferior olivary nucleus, the connection with the cerebellum being made by another neurone system extending from the cerebellum to the olivary nucleus. In the cord these fibres do not form a solid bundle, but are scattered through the fibres of Gowers's tract, the direct cerebellar tract, and the crossed pyramidal tract. Investigators are not in accord as to the exact paths which these fibres follow in passing from the cerebellum to the cord.

III. Schultze's "Comma" Tract of descending fibres in Burdach's column has been already described.

Practical Study.

Transverse Section of the Human Spinal Cord—Dorsal Region. Stained by Weigert's method.

This section was taken from a cord after a lesion which had destroyed the descending fibre tract of one side above the pyramidal decussation.

Dissecting Microscope and Low Power.—Note the areas

of *degeneration* (1) in the dorso-lateral region corresponding to the *crossed pyramidal tract;* (2) much smaller than the preceding, lying on the opposite side of the cord next the anterior median fissure and corresponding to the *direct pyramidal tract.* The lesion above the pyramidal decussation was unilateral. It will be remembered (see page 222) that in the decussation of the pyramids most of the fibres cross over to the opposite postero-lateral region of the cord to become the crossed pyramidal tract. Thus the degeneration, which above the decussation was unilateral, is expressed in the cord by degeneration of the direct tract on the same side as the lesion and of the crossed tract on the opposite side. By means of this section we are able to make out the areas of the direct and of the crossed pyramidal tracts. Combining the two pictures, that seen in the specimen of ascending degeneration and the one just studied, we are able to determine the areas occupied by all the long-fibre systems of the cord. Subtracting these areas from the total area of white matter, the area remaining is that occupied by the already described short-fibre systems of the cord, the fundamental columns or ground bundles.

Make a drawing showing the general topography of the cord and indicate carefully the location and extent of the degenerated areas.

Transverse Section through the Lumbar Spinal Cord of a Human Fœtus of Six and a Half Months. Stained by the method of Weigert. Mounted in **balsam.**

Low Power.—Note the following points: The size and shape of the cord; the shape of the gray matter and its proportion relative to the white matter, as compared with the adult lumbar cord, which you have studied; the plexus of fine fibres throughout the gray matter. What are the sources of these fibres? Note the fibres of the *anterior roots* passing from the anterior horns to the surface of the cord; the entrance of the fibres of the *posterior root*—trace these fibres as far as possible and note what becomes of them; the *anterior commissure* and, as far as possible, the origin and distribution of the fibres of which it is composed; the *substantia gelatinosa of Rolando* and the *substantia gelatinosa centralis;* the *canalis centralis* lined with epithelial cells (ependyma cells)—observe the character of these cells with the high power;

the small number of medullated fibres as compared with the adult cord; the *crossed pyramidal tracts* and that they look lighter than the rest of the white matter—this is seen under the high power to be due to the absence of medullated fibres.

Naked Eye or Dissecting Microscope.—Make a large outline drawing of the cord and of the gray matter.

Low Power.—Fill in the above described details. Indicate the arrangement of cells in the anterior horn—these can usually be separated into a mesial group, a central group, a postero-lateral and an antero-lateral group.

Transverse Section through the Cervical Spinal Cord of a Human Fœtus of Six and a Half Months. Stained by Weigert's method. Mounted in **balsam.**

Low Power.—Compare this section with the preceding. Note the shape and size of the cord, the shape of the gray matter and its relative proportion to the white matter. Note the appearance of the crossed and of the direct pyramidal tracts, and that this appearance is due to the absence in them of medullated nerve fibres. Why are the direct pyramidal tracts visible in this section and not in the preceding?

Make an outline drawing of the specimen showing the above points.

FUNDAMENTAL FIBRES OF THE SPINAL CORD (GROUND BUNDLES).

The tracts which have been described are known as the long-fibre tracts of the spinal cord. It was noted in connection with the last section that, in mapping out the areas in the cord which these long tracts occupy, a considerable portion of the cord remains unaccounted for. This area is especially large in the antero-lateral region, where it is known as the antero-lateral ground bundle. It extends up along the outer side of the posterior horn between the gray matter and the crossed pyramidal tract. A few of these fibres are also found in the posterior column just back of the commissure and extending up along the medial aspect of the horn. Generally speaking, these fibres constitute the short-fibre system

of the cord, serving as *longitudinal commissural fibres* to bring the different segments of the cord into communication. The shorter fibres lie nearest the gray matter and link together adjacent segments. The longer fibres lie farther out and continue through several segments. The fibres which make up these columns have already been seen to be the axones of neurones whose cell bodies and dendrites lie in the gray matter of the cord. These fibres run up and down in the fundamental columns, sending collaterals into the gray matter and themselves terminating there.

THE MEDULLA OBLONGATA.

The *medulla oblongata* is the continuation upward of the spinal cord and extends from the lower limit of the pyramidal decussation below to the lower margin of the pons Varolii above. The length of the medulla is about an inch, and its diameters, which at its lower end correspond to those of the cord, increase from below upward.

Externally, the medulla shows the continuation upward of the anterior and the posterior fissures of the cord, the latter opening at about the middle of the medulla into the cavity of the *fourth ventricle.* On either side of the anterior fissure is a prominence caused by the *anterior pyramid,* and to the outer side of the pyramid the bulging of the *olivary body* may be seen. The antero-lateral surface of the medulla is also marked by the exit of the sixth to the twelfth (inclusive) cranial nerves. The posterior surface shows two prominences on either side: one, next the posterior fissure, known as the *clava,* is caused by the *nucleus funiculi gracilis,* or *nucleus of the column of Goll;* the other, lying just to the outer side of the clava, is due to the *nucleus funiculi cuneati,* or *nucleus of the column of Burdach.*

The internal structure of the medulla considerably resembles that of the cord. This is especially true of the lower part of the medulla, the structures of which are directly continuous with those of the cord. The fibre tracts of the cord, however, assume in the medulla new directions and in so doing break up the formation of the gray matter. This, and the appearance of certain

new masses of gray matter and some new fibre bundles, many of them connected with the cranial nerves, are the main factors determining the difference in structure between cord and medulla.

The *cranial nerves,* with the exception of the *first* (olfactory) and the *second* (optic), are analogous, both embryologically and anatomically, to the spinal nerves.

The motor root fibres of the cranial nerves are the axones of neurones whose cell bodies are situated in the gray matter of the medulla and parts above (motor nuclei of the cranial nerves), just as the motor root fibres of the spinal nerves are the axones of neurones whose cell bodies are situated in the gray matter of the cord (anterior horns). These motor nuclei are the nuclei of origin for these nerves. They are nuclei of termination for neurones of higher systems which serve to bring the peripheral neurone under the control of higher centres.

The neurones which constitute the sensory portions of the cranial nerves have their cell bodies situated in ganglia outside the central nervous system. These ganglia correspond to the posterior root ganglia of the spinal nerves. The outwardly-directed processes of these cells pass to their peripheral terminations, as do those of the spinal ganglia cells. The central axones of these neurones enter the medulla and form longitudinal tracts of fibres in a manner quite analogous to the formation of the posterior columns by the central axones of the spinal ganglia cells. The sensory root fibres of the cranial nerves, however, do not ascend, as do those of the spinal nerves, but turn spinalward, forming descending roots. These fibres terminate in the gray matter of the medulla (terminal nuclei of the cranial nerves) in the same manner as do the spinal sensory root fibres in the gray matter of the cord and medulla.

The internal structure of the medulla can be best appreciated by studying a series of transverse sections.

Practical Study.

Transverse Section of the Medulla through the Decussation of the Pyramids (motor decussation). Stained by Weigert's method.

Note.—The numbers in parenthesis after the words in italics refer to the numbers on the chart.

Dissecting Microscope and Low Power.—Compare section with that through the cervical cord; note the following structures which have been studied in the cord:

1. The *posterior column* (1), the *column of Goll* (funiculus gracilis), and (2) the *column of Burdach* (funiculus cuneatus), remain as in the cervical cord.

2. The *lateral column* (3), the *tract of Gowers* (antero-lateral ascending tract), and the *tract of Flechsig* (direct cerebellar tract) occupy the same positions as in the cervical cord. The crossed pyramidal tract is decreased in size, owing to the fact that fewer fibres have crossed to it from the anterior pyramid (see 8).

3. The *anterior columns* (4), now called the *anterior pyramids*, are increased in size. This is due to the fact that fewer fibres have left them to decussate and enter the crossed pyramidal tracts (see 8).

4. The *posterior horn* (5) is larger, especially the substantia gelatinosa of Rolando, and is almost entirely separated from the rest of the gray matter, being connected with it by a very long, slender cervix.

5. The *anterior horn* (6) is cut off from the rest of the gray matter by decussating pyramidal fibres.

6. The *canalis centralis* and (7) the *substantia gelatinosa centralis* are the same as in the cervical cord.

Note also the following structures which have not been before met with:

7. The *formatio reticularis* (9) begins to show in this section, although not so well developed as higher up in the medulla. Its coarse basket-work appearance is due to a breaking-up of the lateral gray matter by fibres which run longitudinally—mainly, continuations into the medulla of the lateral fundamental column fibres of the cord.

8. *Decussation of the pyramids* (10). This is a most important feature of the section. Bundles of fibres are seen crossing from the anterior pyramid of one side to the opposite dorso-lateral column, where they turn downward as the crossed pyramidal tract. These fibres, as already noted in the cord, are descending axones from motor cells situated in the cerebral cortex. In the pyramidal decussation most of these fibres cross to the opposite postero-lat-

eral region to pass down the cord as the crossed pyramidal tract, while a few remain in their original anterior position to continue down the cord as the direct pyramidal tract. The bundles of fibres do not cross in a transverse plane, but take a downward direction at the same time. For this reason transverse sections show these fibres cut rather obliquely. Because ~~of the~~ the fact that the fibres cross in alternate bundles, the number of decussating fibres is seen to be much greater on one side than on the other.

Make an outline drawing with the *dissecting microscope* and fill in all details of the section with the *low power*.

Transverse Section of the Medulla through the Lower Part of the Sensory Decussation (decussation of the fillet). Stained by the method of Weigert. Mounted in **balsam.**

Dissecting Microscope and Low Power.—Note the following already mentioned structures:

1. The *posterior columns.* Both the column of Goll (1) (funiculus gracilis) and the column of Burdach (2) (funiculus cuneatus) are diminished in size, being shortened dorso-ventrally by two new masses of gray matter, one in the ventral part of each column (see 9).

2. The *lateral column* (3) still contains Gowers's tract (antero-lateral ascending tract) and the direct cerebellar tract (Flechsig) in about the same position as in previous sections. The lateral column is much depleted in size. This is due to the absence of almost all the crossed pyramidal fibres, as this section is at the extreme upper limit of the pyramidal decussation and nearly all of the descending motor fibres are here contained in the anterior pyramids.

3. The *anterior pyramids* (4) are increased in size, containing now nearly all of the descending cerebro-spinal fibres.

4. The *posterior horn* (5) is somewhat larger than in the preceding section.

5. The *anterior horn* (6) is less definite, owing to its being broken up by bundles of longitudinal fibres forming a part of the formatio reticularis.

6. The *canalis centralis* (7) and the *substantia gelatinosa centralis* (8) remain the same.

7. The *formatio reticularis* (9) is considerably more extensive.

8. *The decussation of the pyramids* (10) has almost ceased, although a few fibres may still be seen passing from the anterior pyramid to the opposite dorso-lateral region, and a wedge-shaped mass of fibres decussating in the median line may often be noticed.

The following new structures are to be observed:

9. *The nuclei of the posterior columns.* These occupy the ventral part of the columns and are known respectively as *the nucleus of the column of Goll,* or *the nucleus funiculi gracilis* (12), and *the nucleus of the column of Burdach,* or *the nucleus funiculi cuneati* (13). These nuclei serve as the nuclei of termination for the fibres of the posterior columns. With their termination in these nuclei we come to the ending of that system of fibres which we have traced from their origin in the cells of the spinal ganglia. In other words, we have completed the course of the peripheral sensory neurone (excepting those connected with the cranial nerves). As the fibres of the posterior columns are constantly terminating in these nuclei, there is, in passing from below upward, a constant increase in the size of the nuclei and a corresponding decrease in the size of the posterior columns. By means of neurones whose cell bodies are situated in these nuclei, the sensory conduction path is continued brainward. These neurones may be separated into four systems: *(a)* An uncrossed tract through the restiform body of the same side to the cerebellum. *(b)* A crossed tract through the opposite restiform body to the cerebellum. *(c)* A crossed tract to the optic thalamus. *(d)* A crossed tract to the cerebral cortex. *(c)* and *(d)* form the fillet.

10. *Internal arcuate fibres* (14, *a*). These are seen passing ventrally and inward from the nuclei of the posterior columns to a point just below the central canal, where they form the

11. *Sensory decussation* (14, *b*), or decussation of the fillet. These fibres are axones of neurones whose cell bodies are situated in the nuclei of the posterior columns. After decussating they turn brainward, forming a tract of fibres known as the

12. *Fillet* (14, *c*), or *lemniscus,* and lying just dorsal to the anterior pyramid.

13. *Spinal* (descending) *root* (15) of the *fifth cranial nerve* (trigeminus). This is a bundle of very fine fibres lying just ex-

ternal to the posterior horn, occupying somewhat the position of Lissauer's column in the cord.

Make an outline drawing with the *dissecting microscope* and fill in all the details of the section with the *low power.*

Transverse Section of the Medulla through the Upper Part of the Sensory Decussation (decussation of the fillet). Stained by Weigert's method. Mounted in **balsam.**

Dissecting Microscope and Low Power.—Observe the following structures which have been noted in the preceding section:

1. The *posterior columns;* greatly diminished, their places being now occupied by their respective nuclei. This disappearance of the posterior columns is due to the fact that nearly all their fibres have now terminated in end arborizations around the cells of these nuclei (their nuclei of termination).

2. In the *lateral columns* (3) the tract of Gowers and the direct cerebellar tract occupy about the same positions as in the preceding section. The crossed pyramidal tract has entirely disappeared, all of the descending motor fibres being now in the anterior pyramids.

3. The *anterior pyramids* (11) remain the same.

4. The *posterior horn* (5) is increased in size.

5. The *anterior horn* (6) is almost entirely lost in the formatio reticularis. There usually remains a fairly definite group of cells in the lateral region, which is known as the *lateral nucleus.*

6. The *central canal* (7) is nearer the dorsal surface of the medulla and is surrounded by a larger area of gelatinous substance (8).

7. The *formatio reticularis* (9) is more extensive.

8. The *pyramidal decussation* (10) has entirely ceased, all of its fibres being now in the anterior pyramids.

9. The *nuclei of the posterior columns* (12 and 13) are much larger than in the preceding section, occupying almost all the area previously occupied by the columns themselves.

10. The *internal arcuate fibres* (14, *a*) are more abundant.

11. The *sensory decussation* (14, *b*) is more extensive.

12. The *fillet* (14, *c*) is much larger, as it now contains nearly all the axones from the posterior column nuclei.

13. The *spinal root of the fifth* (15) (trigeminus) *cranial nerve*

is larger, as less fibres have left the root to terminate in the gray matter.

Note also the following new structures:

14. The *accessory olivary nucleus* (16); an elongated L-shaped mass of gray matter lying just dorsal to the anterior pyramids.

15. The *nucleus arciformis* (17) lies on the surface of the medulla, internal and ventral to the anterior pyramid.

16. The *fasciculus solitarius* (18) shows in some of the sections as a distinct round bundle of fibres at the outer edge of the dorsal gray matter. It consists of the *descending* or *sensory root fibres of the ninth (glosso-pharyngeal) and tenth (vagus) cranial nerves.*

17. The *nucleus of origin of the twelfth* (19) *cranial nerve (hypoglossal);* a collection of nerve cells lying in the ventral part of the substantia gelatinosa centralis near the median line. Root fibres (20) of this nerve may be seen passing from this nucleus to the ventral surface of the cord.

18. The *posterior longitudinal fasciculus* (21); a bundle of fibres situated just dorsal to the fillet and not easily differentiated from the latter tract. These fibres are the upward continuation of the anterior ground bundles of the cord.

19. The *external arcuate fibres* (25). These are often present at this level, running parallel to the lateral surface of the cord just under the pia mater. They are axones of neurones whose cell bodies are situated in the nucleus funiculi gracilis and the nucleus funiculi cuneati. These axones pass to the cerebellum through the restiform body. It is probable that some of these fibres enter the nucleus arciformis.

Make an outline drawing with the *dissecting microscope* and fill in the above details with the *low power.*

Transverse Section of the Medulla through the Lower Part of the Olivary Nucleus. Weigert stain. Mounted in **balsam.**

Dissecting Microscope and Low Power.—Note that certain structures which we have been studying have entirely disappeared. These are:

1. The *posterior columns* (1 and 2).

5. The *anterior horn* (6); lost in the formatio reticularis.

6. The *canalis centralis* (7) has opened into the *fourth ventricle,* the substantia gelatinosa centralis forming its floor.

8. *Decussation of the pyramids* (10).

Note the following structures already studied:

2. The *lateral column* (3); still contains Gowers's tract (antero-lateral ascending) and the direct cerebellar tract.

3. The anterior pyramids (11) remain the same.

4. *Posterior horn* is diminished in size and vague (5).

6. The *substantia gelatinosa centralis* (8) now lies in the floor of the fourth ventricle.

7. The *formatio reticularis* occupies an increased area (9).

9. The *nuclei of the posterior columns* (12 and 13) are much diminished in size and not clearly defined.

10. The *internal arcuate fibres* (14, *a*) are more numerous.

11. The *sensory decussation* (14, *b*), or decussation of the fillet, is more extended dorso-ventrally, forming the median raphé.

12. The *fillet,* or *lemniscus* (14, *c*), is larger, more fibres having entered it through its decussation.

13. The *spinal root of the fifth nerve* (trigeminus) (15) is larger, less fibres having left it to terminate in the gray matter.

14. The *accessory olivary nucleus* (16) is larger, lying along the external margins of the fillet and pyramid. It is separated from the main body of the olive by the root fibres of the twelfth cranial nerve.

15. The *nucleus arciformis* (17) is usually present. This nucleus may serve as a terminal nucleus for some of the external arcuate fibres.

16. *Fasciculus solitarius* (18), or descending sensory root fibres of the ninth (glosso-pharyngeal) and the tenth (vagus) nerves, is larger, fewer fibres having left it to terminate in the gray matter.

17. The *nucleus of origin of the twelfth cranial nerve* (hypoglossal) (19) is larger, and distinct bundles of fibres (root fibres of the twelfth cranial nerve) (20) may be traced from the nucleus passing ventrally and outward to the surface between the accessory olivary nucleus and the main body of the olive.

18. The *posterior longitudinal fasciculus* (21) occupies the same position.

19. *External arcuate fibres* (25) are more numerous than in the preceding section.

The following new structures are to be noted:

20. The *olivary nucleus* (23), an irregularly convoluted mass of gray matter above and to the outer side of the anterior pyramid. Note the fibres which pass as internal arcuate fibres, through the median raphé, into the restiform body. These fibres ultimately pass to the opposite side of the cerebellum (cerebello-olivary fibres). Some of these latter are probably ascending axones of cells in the olivary nucleus; others are probably descending axones from cells in the cerebellum. Fibre tracts also connect the olives and the cord, passing through the ventral and lateral ground bundles. It is uncertain whether these are descending or ascending fibres, or both.

21. *Corpus restiforme,* or *inferior cerebellar peduncle* (24). This is just beginning to form at the upper and outer angle of the medulla. Its relation to the opposite olive has already been noted.

22. The *fourth ventricle* (22) now lies over the medulla, the canalis centralis having opened up into the cavity of the ventricle and the substantia gelatinosa centralis being spread out on its floor.

Make an outline drawing with the *dissecting microscope* and fill in the above details with the *low power.*

Transverse Section of the Medulla through the Middle of the Olivary Nucleus. Weigert stain. Mounted in **balsam.**

Dissecting Microscope and Low Power.—The following structures present in the last section have disappeared:

9. The *nuclei of the posterior columns* (12 and 13).

11. The *sensory decussation* (14, *b*).

Note the following structures already studied:

2. The *lateral column* (3). The direct cerebellar tract is now a part of the corpus restiforme.

3. The *anterior pyramids* remain the same (11).

4. The *remains* of the *posterior horn* (5) are still visible, though smaller and more vague.

6. The substantia gelatinosa centralis (8), in the floor of the fourth ventricle.

7. The *formatio reticularis* is increased in size (9).

10. The *internal arcuate fibres* (14, *a*) are diminished in number and are no longer derived from the nuclei of the posterior columns. As they cross the median line, these fibres still form the raphé.

12. The *fillet,* or *lemniscus* (14, *c*), is now completely formed and is much extended dorso-ventrally. While the fillet must be considered as the main continuation brainward of the spinal sensory conduction path, other fibres enter into its formation. Thus we find in the fillet axones of cells situated in the formatio reticularis of the medulla and of the pons, also axones from the nuclei of termination of the sensory cranial nerves. The termination of the fillet is also very complex. Though the majority of its fibres terminate in the nuclei of the thalamus, some may pass directly to the cerebral cortex, while still others end in the gray matter of the medulla, pons, mid-brain, and hypothalamic region.

13. The *spinal root of the fifth nerve* (15) is larger, for the same reason as in the last section.

14. The *accessory olive* (16) may be present or absent. There may be a dorsal accessory olive just above the inner end of the main olivary nucleus.

15. The *arciform nucleus* (17) is usually present.

16. The *fasciculus solitarius* (18) is larger and more distinct. In some sections it may be less distinct. In some sections some of its fibres may be seen passing out as sensory root fibres of the ninth and tenth nerves.

17. The *nucleus of origin of the twelfth cranial nerve* (hypoglossus) (19) is diminished in size and only a few of its root fibres can be seen.

18. The *posterior longitudinal fasciculus* (21) is the same as in the preceding section.

19. *External arcuate fibres* (25) may be seen running parallel to the surface of the medulla just under the pia mater.

20. The *olivary nucleus* (23) is larger and more fibres can be seen passing from it to the opposite restiform body (cerebello-olivary fibres).

21. The *corpus restiforme,* or *inferior cerebellar peduncle* (24), is much increased in size. It contains fibres from the nuclei of the posterior columns of both the same and the opposite sides (through both internal and external arcuate fibres); fibres con-

necting the olivary nucleus with the cerebellum; fibres which represent the continuation upward of the direct cerebellar tract.

22. The *fourth ventricle* is more widely open.

Note the following new structures:

23. The *sensory (terminal) nucleus of the ninth (glosso-pharyngeal) and tenth (vagus) cranial nerves* (27); a group of cells lying just to the outer side of the nucleus of the twelfth nerve. The dorsal part of the nucleus belongs to the ninth, the ventral to the tenth nerve.

24. The *nucleus ambiguus* (28), motor nucleus of the ninth and tenth cranial nerves; often difficult to distinguish; lies in the formatio reticularis, about midway between the floor of the ventricle and the inner end of the olivary nucleus, and between the root fibres of the twelfth and tenth cranial nerves. From the cells of this nucleus fibres pass upward to just below the sensory nuclei of their nerves, where they turn sharply downward and outward, joining the sensory root fibres.

25. The *descending* or *spinal root of the vestibular branch of the eighth nerve* (auditory) lies just to the medial side of the restiform body.

Make an outline drawing of the specimen with the naked eye or *dissecting microscope* and fill in the details described above with the *low power.*

Transverse Section of the Medulla through the Upper Part of the Olivary Nucleus and Exit of the Eighth Cranial Nerve. Stained by the method of Weigert. Mounted in **balsam.**

Dissecting Microscope and Low Power.—The following structures seen in the last section have disappeared:

14. The *accessory olivary nucleus* (16), although a small dorsal olivary nucleus may be present.

16. The *fasciculus solitarius* (18), having entirely passed out in the sensory root of the ninth and tenth nerves.

17. The *nucleus of the twelfth cranial* (hypoglossus) nerve (19).

24. The *nucleus ambiguus* (28)—motor nucleus of the ninth and tenth cranial nerves (glosso-pharyngeal and vagus).

The following already observed structures are still present:

2. The remains of the *lateral columns* (3).

3. The *anterior pyramids* (11).

4. The remains of the *posterior horn* (5) may still be seen, but are vague.

6. The *substantia gelatinosa centralis* (8), in the floor of the fourth ventricle.

7. The *formatio reticularis* (9).

10. *Internal arcuate fibres* (14, *a*).

12. The *fillet* (14, *c*) remains the same.

13. The *spinal root of the fifth cranial nerve* (trigeminus) (15) is somewhat increased in size.

15. The *arciform nucleus* (17) may or may not be present.

18. The *posterior longitudinal fasciculus* (21) is usually more distinct.

19. Some *external arcuate fibres* (25) are usually present.

20. The *olivary nucleus* (23) may be about the same size as in the last section, may be larger or much smaller, depending upon how near the upper limit of the nucleus the section is taken.

21. The *restiform body* (24) is much larger, being now almost completely formed. If the roof of the fourth ventricle and a part of the cerebellum be included in the section, the restiform body can be seen passing into the cerebellum as its inferior peduncle.

22. The *fourth ventricle* (22) remains the same.

23. The *sensory* (terminal) *nucleus* (27) *of the ninth and tenth cranial nerves* (glosso-pharyngeal and vagus) may still occasionally be seen. It is not present in the higher sections.

The following new structures are to be noted:

25. The *root fibres of the eighth cranial nerve* (auditory) (30), and its *nuclei* (31) (most important feature of the section). The auditory nerve is divided into two divisions: (1) the *nervus cochleæ* and (2) the *nervus vestibuli.* The fibres of the nervus cochleæ are the axones of bipolar cells in the ganglion spirale or ganglion of Corti. The peripheral processes of these cells end among the epithelial cells of Corti's organ. The central processes of these cells enter the medulla at the junction of the medulla and pons as the cochlear root of the auditory nerve, to terminate in arborizations among the cells of the nucleus nervi cochleæ ventralis and the nucleus nervi cochleæ dorsalis. The former is the larger of the two nuclei and can be seen in the section to the outer side of the

entering fibres. Some bundles of fibres pass this nucleus to end in the dorsal nucleus of the cochlear nerve, or nucleus of the tuberculum acusticum (33). This nucleus may or may not show in the section. By means of neurones whose cell bodies are situated in these nuclei, the auditory impressions are carried to higher centres. The neurones of the nervus vestibuli, or vestibular portion of the auditory nerve, have their cell bodies situated in Scarpa's ganglion or the ganglion vestibulare. These cells are bipolar, their peripheral processes ending freely among the hair cells of the crista and macula acustica, their central processes forming a bundle which can be seen in the section entering the medulla just to the inner side of the cochlear nerve, and forming the vestibular root of the auditory nerve. Fibres of this root can be seen passing to two terminal nuclei: one (Deiter's nucleus, or *nucleus nervi vestibuli lateralis)* is situated at the end of the main bundle of fibres; the other (nucleus of von Bechterew, or *nucleus nervi vestibuli superior)* is situated just dorsal to Deiter's nucleus in the lateral wall of the fourth ventricle. Other fibres of the nervus vestibuli pass to the nucleus nervi vestibuli medialis and by means of a descending or spinal root (seen in the previous section) to the *nucleus nervi vestibuli spinalis.* These two nuclei can be seen in some of the lower sections of this level and in some of the higher sections of the preceding level. The median nucleus lies just to the inner side of the position which a little higher up is occupied by Deiter's nucleus. The descending root and its nucleus lie just ventral and external to the medial nucleus, or just to the outer side of the fasciculus solitarius and its nucleus (if the latter can be seen in the section).

26. The *root fibres of the seventh* (facial) *cranial nerve* (38) and *their nucleus* (39). This nucleus (nucleus of origin) consists of a fairly well defined group of large motor cells situated deep in the formatio reticularis.

The axones of the cells of this nucleus pass dorsally and medially toward the floor of the fourth ventricle. Here they turn and ascend in the floor of the fourth ventricle—these appear in sections as transversely cut fibres—to the genu, or bend, where they turn and form a compact bundle which passes ventrolaterally to the surface.

Only portions of this course of the root fibres of this nerve can be seen in any one section.

27. The *nucleus* (40) (nucleus of origin) *of the sixth cranial nerve*—abducens—consists of a group of large motor cells, lying in the floor of the fourth ventricle, partially surrounded by the genu of the seventh nerve. From this nucleus fibres (37) may be seen passing to the surface through the formatio reticularis medial to the superior olivary nucleus.

28. *Striæ acusticæ* (34) crossing the floor of the fourth ventricle. Some of these fibres connect the dorsal nucleus of the cochlear nerve with higher centres.

29. Fibres of the *pons Varolii* (35) may be seen in some of the highest sections just ventral to the pyramids.

Make an outline drawing of the section with the *naked eye or dissection microscope* and fill in all details with the *low power.*

THE PONS VAROLII.

The pons consists of two distinct parts: *(a)* a *ventral part,* or *pons proper,* mainly transverse fibres, their nuclei, and, intermingled with these, longitudinal fibres; and *(b)* a *dorsal part,* or *tegmentum,* comprising all structures ventral to the fourth ventricle and dorsal to the transverse fibres of the pons proper.

Transverse Section of the Pons through the Exit of the Root Fibres of the Fifth Cranial Nerve (trigeminus). Stained by the method of Weigert.

Dissecting Microscope and Low Power.—The following structures seen in the last section have disappeared:

2. The *lateral columns* (3).

10. The *internal arcuate fibres* (14, *a*).

15. The *nucleus arciformis* (17).

19. The *external arcuate fibres* (25).

20. The *olivary nucleus* (23).

21. The *restiform body* (24); has passed into the cerebellum.

23. The *sensory nucleus* of the ninth and tenth cranial nerves (27).

25. The *root fibres* of the eighth cranial nerve and their *nuclei* (31, 32).

26. The *nucleus* (39) and *root fibres* (38) of the seventh cranial nerve.

27. The *nucleus* (40) and *root fibres* (37) of the sixth cranial nerve.

28. *Striæ acusticæ* (34).

The following already studied structures are to be noted:

3. The *anterior pyramids* (11) are now broken up into bundles of fibres which pass longitudinally and can be seen in transverse section among the transversely passing pontile fibres. Some of these cross-cut fibres do not represent pyramidal fibres, but fibres connecting the nuclei pontis with the cerebrum, and possibly some longitudinal commissural fibres connecting the nuclei pontis.

7. The *formatio reticularis* (9) occupies a considerable portion of the dorsal part of the pons, its gray matter being known as the nucleus of the formatio reticularis.

11. The *median raphé.*

12. The *fillet* (14, *c*)—now often called the *medial lemniscus* to distinguish it from the *lateral lemniscus* (see 31)—is much flattened dorso-ventrally and broken up into several bundles of fibres which lie just ventral to the formatio reticularis and dorsal to the most dorsal transverse pontile fibres.

18. The *posterior longitudinal fasciculus* (21) lies dorso-medial to the formatio reticularis quite close to the floor of the fourth ventricle.

22. The *fourth ventricle* (22) is narrowing down as it approaches the iter.

29. *Transverse pontile fibres* (35), or middle peduncle of the cerebellum, connect the nuclei pontis with the opposite cerebellar hemisphere. They are divided by longitudinally running bundles (see 3) into superficial transverse fibres and deep transverse fibres.

The following new structures are to be noted:

30. The *nuclei pontis* (36); masses of gray matter lying among the fibres of the pons (see 3 and 29). They are nuclei of origin of the transverse pontile fibres.

31. The *lateral lemniscus* (43), or lateral fillet, lies to the outer side of the ventral part of the formatio reticularis. It contains a mass of gray matter known as the nucleus of the lateral lemniscus. Its fibres are mainly a *secondary acustic tract* or axones from cells

lying in the terminal nuclei of the cochlear nerve. It also contains fibres from the nucleus of the lateral lemniscus and probably some fibres from the olivary nucleus. The fibres of the lateral lemniscus terminate mainly in the gray matter of the anterior and posterior corpora quadrigemina, most of them in the corpora quadrigemina of the same side, a few in the corpora quadrigemina of the opposite side. Some fibres of the lateral lemniscus probably pass both anterior and posterior corpora quadrigemina to end in the cortex of the temporal lobe.

32. The *brachium conjunctivum,* or superior peduncle of the cerebellum (44), occupies a crescentic area lateral to the fourth ventricle.

33. *Root fibres* of the fifth cranial nerve (trigeminus) breaking through the fibres of the pons.

This root consists of two parts, a large posterior *sensory* part and a smaller anterior *motor part.*

(1) *Sensory root.* The cell bodies of the neurones whose axones make up the sensory root of the fifth nerve are situated in the Gasserian ganglion, or ganglion semilunaire. This ganglion is analogous to the posterior root ganglion of the spinal nerve. These cells are unipolar, the single process bifurcating as in the cells of the spinal ganglia. Their peripheral branches pass to the surface. Their central branches pierce the fibres of the pons and, reaching the floor of the fourth ventricle, bifurcate. The short ascending arms terminate in the main sensory nucleus of the fifth nerve in the substantia gelatinosa. The long descending arms form the descending or spinal root of the fifth nerve, which has been observed in all levels of the medulla above the pyramidal decussation. The fibres of this root send collaterals into, and terminate in, the substantia gelatinosa, which thus constitutes an extended series of nuclei for this root.

(2) *Motor root.* The cell bodies of the neurones whose axones constitute the motor root of the fifth nerve are situated in two nuclei. One of these, the principal motor nucleus of the fifth nerve, is located in the dorsal part of the pons just anterior to the nuclei of the sixth and seventh nerves and to the medial side of the main mass of fifth root fibres. The smaller nucleus consists of a long column of cells extending from this level upward to the

region of the corpora quadrigemina. The axones from this nucleus form the descending motor or mesencephalic root of the fifth nerve. The axones from these two nuclei join to form the motor root of the fifth nerve.

34. The *descending motor* or mesencephalic root of the fifth nerve (49), lying in the lateral wall of the fourth ventricle.

35. The *substantia ferruginea;* a group of pigmented nerve cells lying in the outer part of the gray matter of the floor of the fourth ventricle.

36. The *valve of Vieussens,* or anterior medullary velum, forming at this level the root of the fourth ventricle. Note that it is composed of both gray matter and white matter.

Make an outline drawing of the section, using the *naked eye* or *dissecting microscope,* and fill in the above described details with the *low power.*

LEVEL OF THE CORPORA QUADRIGEMINA.

Transverse Section through the Posterior Corpora Quadrigemina. Stained by Weigert's method. Mounted in **balsam.**

Dissecting Microscope and Low Power.—Note that the following structures have disappeared:

22. The *fourth ventricle* (22); now become the iter.

33. The *root fibres* of the fifth cranial nerve.

35. The *substantia ferruginea,* or locus ceruleus.

Note the following already studied structures:

3. The *anterior pyramids* (11); still more broken up into separate bundles of fibres. The number of longitudinally running fibres (cut transversely in the section) is seen to be much greater than the number of fibres which have been seen making up the pyramids. These new fibres, which it is impossible to distinguish from the pyramidal fibres, are mainly fibres connecting the nuclei pontis with the cerebrum.

6. The *substantia gelatinosa centralis* (8) in the floor of the iter.

7. The *formatio reticularis* (9).

11. The *median raphé.*

12. The *fillet,* or median lemniscus (14, *c*); much flattened

dorso-ventrally and situated just dorsal to the transverse pontile fibres.

18. The *posterior longitudinal fasciculus* (21) is now a distinctly separate bundle.

29. The *transverse pontile fibres* are still extensive (35).

30. The *nuclei pontis* (36) are prominent features among the pons fibres.

31. The *lateral lemniscus* (43) and its *nucleus* are seen in the dorso-lateral region.

32. The *superior cerebellar peduncles,* or brachia conjunctiva (44); these may be seen decussating just ventral to the iter. They are composed mainly of axones the cell bodies of which are situated in the dentate nuclei of the cerebellum. Crossing the median line they form the decussation of the superior peduncles, or decussatio brachii conjunctivi, and terminate for the most part in the *red nuclei* (see 44). Other fibres of the superior peduncle appear to be axones of cells situated in other cerebellar nuclei (nucleus fastigii, nucleus globosus, and nucleus emboliformis) and in the cerebellar cortex. These fibres also terminate mainly in the red nucleus of the opposite side. There are probably also a few fibres in the superior peduncles which are axones of cells located in higher centres and which terminate in the cerebellum.

34. The *descending motor* or *mesencephalic root* (49) of the fifth nerve. In some sections the nucleus (51) of this root may be seen. It is usually not very distinct.

Note the following new structures:

39. The *posterior corpora quadrigemina* (47). These consist mainly of gray matter and are connected with parts above and below by tracts of fibres. The fibres which ascend to terminate in the gray matter of the posterior corpus quadrigeminum come mainly from the lateral lemniscus (for fibres which this contains see 31, page 240). From the cells of the gray matter of the posterior corpus quadrigeminum some axones descend in the lateral lemniscus; other axones ascend, joining the fibres of that part of the lateral lemniscus which passes by the posterior corpus quadrigeminum. These together form the brachium quadrigeminum inferius and pass to the anterior corpus quadrigeminum and to the corpus geniculatum mediale.

40. The *iter a tertio ad quartum ventriculum,* also called the *aqueduct of Sylvius* or the aqueductus cerebri (48); connects the fourth ventricle with the third ventricle.

41. *Root fibres* of the fourth cranial nerve (pathetic) (50). These will show only in the lower sections of this set. These fibres are axones of a group of cells which lies deep in the floor of the iter. These axones pass first dorso-laterally to about the position of the descending root of the fifth nerve. They then turn and run spinalward. At the level of the anterior medullary velum they turn dorso-mesially and form a decussation in the substance of the velum with similar axones from the opposite side. After decussating, these axones, as the root of the fourth nerve, pierce the dorsal surface just posterior to the posterior corpora quadrigemina, skirt the lateral surface of the cerebral peduncles, and appear on the ventral surface just anterior to the pons.

Make an outline drawing with the ***naked eye*** or *dissecting microscope* and fill in the above-described details with the *low power.*

Transverse Section through the Anterior Corpora Quadrigemina. Stained by Weigert's method. Mounted in **balsam**.

Naked Eye.—Note the marked change in the *shape* of the section. This is due mainly to the absence of the transverse pontile fibres and to the deep groove which has appeared anteriorly between the diverging cerebral peduncles.

Dissecting Microscope and Low Power.—Note the disappearance of the following structures present in the last section:

29. The *transverse fibres of the pons* (35).

30. The *nuclei pontis* (36).

34. The *descending* or mesencephalic root of the *fifth nerve* (49).

39. The *posterior corpora quadrigemina* (47).

41. *Root fibres* of the *fourth cranial nerve* and their *nucleus* (50).

Note the following already studied structures:

3. The *anterior pyramids* (11). These have now passed into the cerebral peduncles, where they form the middle fibres of the ventral part of the crusta. The fibres which have been seen as the

longitudinal pontile fibres connecting the nuclei pontis with the cerebrum have all become a part of the cerebral peduncles.

6. The *substantia gelatinosa centralis* (8).

7. The *formatio reticularis* (9); less extensive.

12. The *fillet,* or median lemniscus (43). Part of the fillet fibres has passed into the crusta. Some of the fibres still remain near the lateral surface just dorsal to the substantia nigra (57).

18. The *posterior longitudinal fasciculus* (21) is usually somewhat smaller than in the preceding section. It lies just ventral to the nucleus of origin of the third cranial nerve (55).

31. The *lateral lemniscus* (43). This is reduced in size from loss of some fibres which ended in the posterior corpus quadrigeminum.

32. The *superior cerebellar peduncles* (44) can be seen as longitudinally running fibres connected with the nucleus ruber (58) in which its fibres are terminating.

40. The *iter* (48) remains the same.

The following new structures are to be noted:

42. The *anterior corpora quadrigemina* (53); consist of both gray matter and white matter. The white matter is made up mainly of fibres of the *optic tracts,* axones of neurones whose cell bodies are located in the retinæ. The gray matter of the anterior corpora quadrigemina serves as the terminal nuclei for these axones. They also serve as terminal nuclei for some of the axones of the lateral lemniscus, *i.e.,* for the *secondary acoustic tract.* The neurones whose cell bodies are located in these nuclei send their axones downward in two tracts, one of which terminates in the nuclei pontis and thus brings the superior corpora quadrigemina into connection with the opposite cerebellar hemisphere; the other probably terminates in the motor nuclei of the cranial nerves.

43. The *crura cerebri,* or cerebral peduncles (56), composed of two parts, a ventral part of longitudinally running fibres and a dorsal part of gray matter. The gray matter consists of cells which are deeply *pigmented* and give a dark appearance to the part when seen unstained. It is known as the *substantia nigra* (57). The destination of the axones of these cells is unknown. The white matter of the crus, or pes pedunculi, consists of the continuation upward of the bundles of fibres which have been seen

running longitudinally among the transverse fibres of the pons. These, it will be remembered, consisted of the pyramidal fibre tract and of fibres from the nuclei pontis to the cerebrum. It is probable that many of these fibres send collaterals in among the cells of the substantia nigra.

44. The *nucleus ruber,* or red nucleus (58). A large mass of gray matter lying between the substantia nigra and the posterior longitudinal fasciculus just to the outer side of the root fibres of the third nerve. The relation of this nucleus to the superior peduncles of the cerebellum was described in connection with the preceding section. From cells in this nucleus axones pass upward to connect it with higher centres and downward to bring the nucleus into connection with the spinal cord.

45. The *root fibres of the third cranial nerve* (oculo-motor) (54). These fibres are seen to take origin in a group of large motor cells (55) situated rather deep in the gray matter ventral to the aqueduct of Sylvius. From this nucleus bundles of fibres may be seen passing in a curved course through the formatio reticularis to reach the surface just to the inner side of the crusta.

The following structures may show in some of the sections:

46. The *corpus geniculatum mediale* (59) and the *corpus geniculatum laterale* (60) are two masses of gray matter lying just dorsal to the outer part of the crusta. As each optic tract passes backward it divides, one portion going to the corpus geniculatum mediale, the other to the corpus geniculatum laterale. These gray masses serve as terminal nuclei for the fibres of this tract, their cell bodies being located in the retina. Connecting the geniculate bodies with the corpora quadrigemina may sometimes be seen the brachium quadrigeminum superius and the brachium quadrigeminum inferius.

47. The *optic tract* (61). Portions of this may show in some of the higher sections. It is usually to be seen just to the outer side of the crusta. The fibres of this tract terminate in the geniculate bodies, in the pulvinar, and in the corpora quadrigemina.

48. The *pulvinar* (62) is a collection of gray matter dorsal to the geniculate bodies. In it terminate some of the fibres of the optic tract.

Make an outline drawing with the *naked eye* or *dissecting*

microscope and fill in the above-described details with the *low power.*

THE CEREBRAL PEDUNCLES (CRURA CEREBRI).

Each cerebral peduncle, or crus cerebri, consists of a ventral portion or crusta, a dorsal portion or tegmentum, and, between these, a mass of gray matter, the substantia nigra. The dorsal part of the cerebral peduncle, or tegmentum, represents the continuation brainward of the main sensory tract to the cortex cerebri. Of the ventral part of the peduncles, or crusta, about the middle three-fifths are taken up by the fibres of the pyramidal system (including fibres to the motor nuclei of the cranial nerves). Medial to these fibres in the peduncle are the fibres which pass from the frontal lobe to the nuclei pontis, while external to the pyramidal fibres are the fibres connecting the temporal lobe with the nuclei pontis. These cerebro-pontal fibres have been already noticed as longitudinal fibres of the pons. As the peduncles approach the basal ganglia the substantia nigra disappears and the tegmentum lies just dorsal to the crusta. These bundles of fibres pass through the basal ganglia between the nucleus caudatus and the optic thalamus on the mesial side and the nucleus lenticularis on the lateral side. Here they form the *internal capsule,* which is directly continuous above with the *corona radiata* through which the fibres enter the cortex cerebri. In a horizontal section through the basal ganglia, the internal capsule is seen to present a sharp *bend* or *genu* somewhat anterior to its mid-point. This bend divides the capsule into an anterior portion and a posterior portion. The anterior portion lies between the caudate nucleus internally and the lenticular nucleus externally. This part of the capsule consists mainly of fibres which connect the cortex cerebri and the optic thalamus. The posterior portion of the internal capsule lies between the lenticular nucleus on its outer side and the optic thalamus on its inner side. About the anterior two-thirds of this portion is occupied by the fibres of the pyramidal tract (including descending fibres to the motor cranial nerve nuclei). This tract has now been traced through the *cord, medulla, pons, mid-brain,*

and *internal capsule* to the *cortex cerebri,* in the cells of which the tract originates. The *cortico-pontal* fibres connecting the cortex cerebri with the nuclei pontis, and which have been already noticed as longitudinal pontile fibres, pass through the internal capsule in two separate bundles: one bundle, coming from the frontal lobe, passes through the region of the genu just in front of the main motor tract; the other bundle, coming from the temporal lobe, passes through the posterior part of the internal capsule just behind the pyramidal tract. Through the posterior portion of the internal capsule also passes the continuation upward of the tegmentum, or *main sensory tract* to the cortex.

GENERAL HISTOLOGY OF THE CEREBELLUM.

The cerebellum consists of a central *portion* or *core* of white matter which extends outward into the cortex as a series of transversely disposed branching plates. These, covered by a layer of gray matter, form the *laminæ* which can be seen on the surface, and which on transverse section present the characteristic leaf-like appearance known as the *"arbor vitæ."*

Each leaflet is seen on section to consist of (1) a *central core of white matter* and (2) a covering of *gray matter* which consists of three layers: *(a)* an *internal* or *granular layer, (b)* an *external* or *molecular layer, and* between these *(c)* a layer composed of a single row of very large cells, the *layer of Purkinje cells.*

(1) The *white matter* consists of medullated nerve fibres which pass out in a radial manner into the layers of gray matter. These fibres, while apparently alike, may be subdivided into *(a)* fibres which are axones of cells situated in other parts of the nervous system—these axones are passing to their terminations in the cerebellar cortex; *(b)* fibres which are axones of cells situated in the cerebellum (mainly axones of cells of Purkinje)—these axones pass through the white matter of the cerebellum to terminate in some other part of the nervous system; *(c)* some fibres which are axones of neurones entirely confined to the cerebellum.

(2) The *gray matter,* or *cortex cerebelli,* may be subdivided as follows: *(a)* The *internal, granular,* or *nuclear layer* appears under ordinary staining methods to be composed of a

mass of small, closely packed cells consisting of a nucleus surrounded by a small amount of protoplasm. Intermingled with these cells are medullated and non-medullated nerve fibres. Studied by the method of Golgi, the cerebellar elements of this layer can be divided into (1) *small granule cells* and (2) *large granule cells.* The small granule cells are multipolar, their short dendritic processes ramifying in the granule layer; their axones, which are non-medullated, passing into the molecular layer. Here each axone bifurcates, the branches running parallel to the surface and to the laminæ, and terminating freely. The large granule cells are also multipolar. Their dendrites, however, pass outward to ramify in the molecular layer, while their axones branch rapidly and form a dense network in the granule layer. The dense plexus of nerve fibres in the granule layer is formed by the processes of the cells above described, by collaterals of axones of Purkinje cells, and by fibres which continue into the central core of white matter. Reaching the boundary between the granule layer and the molecular layer, many of these fibres turn and pass horizontally and in a direction transverse to the long axis of the convolution. From these branches pass vertically into the molecular layer. *(b)* The *molecular layer* contains larger and smaller multipolar cells. Most of the dendrites of these cells pass toward the surface. The axones run horizontally in the transverse axis of the convolutions. A few collaterals pass upward. Most of the collaterals and terminals pass downward to end in basket-like arborizations around the bodies of the Purkinje cells. For this reason these cells of the molecular layer are often called "basket cells." There are also found in this layer cells the destination of whose axones is unknown. The fibres of this layer consist of processes of already described cerebellar cells together with fibres which come from the white matter, lose their medullary sheaths, and end in terminal arborizations around the dendritic processes of the Purkinje cells. *(c)* The *cells of Purkinje* lie in the molecular layer just at the margin of the granular layer. From the neck of the cell pass off two large dendritic processes which give rise to an enormous number of branches. These ramify in the molecular layer. This ramification is not equally extensive in all directions, but is much greater in the plane transverse to the long axis of the lamina.

Practical Study.

Section of the Human Cerebellum cut Transversely to the Laminæ. Stained with picro-acid fuchsin. Mount in **balsam.**

Naked Eye and Dissecting Microscope.—Note the general shape and arrangement of the *laminæ* (arbor vitæ). Note that each lamina contains (1) a *central core* of white matter stained a light yellow; (2) outside of this a layer stained a deep red, the *granular layer;* and (3) an external, more lightly stained layer, the *molecular layer.* These last two layers constitute the gray matter, or cortex cerebelli.

Make a drawing showing the above-described features.

Low Power and High Power.—(1) The white matter. Observe that the white matter is composed of parallel fibres. These are stained a light yellow and radiate into the cortex. (2) The gray matter, or cortex. Note *(a)* that the granular layer is composed mainly of small cells, the relatively large nuclei of which are surrounded by a small amount of protoplasm (no cell body at all may be visible). Among these are scattered a few larger cells. *(b)* That between this layer and the molecular layer lie the Purkinje cells. The dendrites of these cells can be seen ramifying in the molecular layer. If possible find where an axone is given off from the lower part or base of one of these cells. *(c)* That the superficial or molecular layer contains the dendrites of the Purkinje cells, a few scattered nerve cells, and many fine fibres which, cut transversely, give the layer a finely granular appearance.

Note the pia mater covering the surface of the convolutions.

Make a drawing of a thin vertical section through the cortex and including some of the white matter, showing accurately the above details.

Section of the Human Cerebellum cut Transversely to the Laminæ. Stained by the method of Nissl. Mount in **balsam.**

Low Power and High Power.—Note the same division into layers as seen in the last section.

High Power.—Make the following drawings: *(a)* One or two Purkinje cells. These are somatochrome cells, *i.e.,* cells in which both nuclei and cytoplasm are stained by Nissl's method. Indicate

carefully the arrangement of the chromophilic bodies. Note whether their arrangement differs from that in the ventral horn cells. *(b)* Several of the cells of the granule layer. These belong to Nissl's class of caryochromes, or cells in which only the nucleus is stained by this method. The cell bodies, being unstained, cannot be distinguished.

Section of the Human Cerebellum cut Transversely to the Laminæ. Stained by the method of Weigert. Mount in **balsam.**

Naked Eye and Dissecting Microscope.—Compare the general appearance of the section with that of the section stained with picro-acid fuchsin.

Low Power.—Note the fibres of the white matter and that they pass out in a radiating manner through the granular layer. Observe the plexus formed by these fibres among the bodies of the Purkinje cells. Remember that as the method of Weigert stains only the medullary sheath, these fibres can be traced only as far as they are medullated, their non-medullated terminations being invisible.

Make a drawing of a single lamina showing the radiation of the medullated fibres through the granular layer and around the cells of Purkinje.

GENERAL HISTOLOGY OF THE CEREBRUM.

Each *cerebral convolution,* like the convolutions of the cerebellum, consists of a *central white core* covered over by a layer of *gray matter,* which latter constitutes the cortex cerebri.

The cortex cerebri may be divided into three fairly distinct layers: *(a)* an *outer, barren,* or *molecular layer* or layer of few nerve cells, *(b)* a *middle layer,* or *layer of pyramidal cells,* and *(c)* an *inner layer,* or *layer of polymorphous cells.*

(a) The barren or molecular layer. The nerve cells of this layer are known as the *cells of Cajal.* They are fusiform, triangular, or irregular in shape, and both their dendrites and axones ramify in this outer layer, the axones passing mainly in a direction parallel to the surface. This layer also contains the terminations of the apical dendrites of the pyramidal cells, some medullated

nerve fibres running parallel to the surface and known as the *superficial tangential fibres,* and a rich plexus of neuroglia.

(b) The layer of pyramidal cells. This is often described as two separate layers, an outer layer of *small pyramidal cells* and a deeper layer of *large pyramidal cells.* It seems better to describe it as a single layer composed mainly of *small pyramidal cells,* in the deeper portion of which the large pyramidal cells are found. Each pyramidal cell has passing off from its outwardly directed angle a large apical or main dendrite. This dendrite sends off small lateral twigs and terminates in numerous branches in the molecular layer. Smaller dendritic processes pass off from the sides and base of the cell. The axone originates from the base of the cell and enters the white matter of the corona radiata. During its passage through the gray matter it sends off collateral branches. Some of these collateral branches are medullated and form the *deep tangential fibres.* The large, medium size, and small cells are apparently identical in structure, differing from one another mainly in size. Among the deeper cells of this layer are found some very large pyramidal cells, called the *cells of Betz.* These cells are found only in the motor cortex, and it is believed that it is the axones of these cells which pass down through the internal capsule to the cord as the main cortico-spinal motor tract.

In this layer are also found cells *(cells of Martinotti)* the dendrites of which pass downward, while their axones pass upward to the molecular layer, where they turn and run parallel to the surface as the (medullated) superficial tangential fibres.

Cells of Golgi type II are also found in this layer. Their axones branch rapidly and end in the gray matter in the vicinity of their cells of origin.

The fibres of this layer consist of the axones and dendrites of cells above described (some axones being medullated) and of axones from cells in other regions which are passing to their terminations (many of these latter being medullated).

(c) The cells of the third layer are fusiform or irregular (polymorphous) in shape. They have no apical dendrites, their protoplasmic processes coming off irregularly and ramifying mainly in this layer. Their axones pass downward into the corona radiata.

The fibres of this layer consist of the axones and dendrites of the

cells found in this layer, of the axones of the pyramidal cells (now mostly medullated), and of axones of cells in other parts of the nervous system which are passing to their terminations (most of these axones are medullated).

The Corona Radiata, the central core of white matter radiating out into the gray matter, thus consists of:

(1) The descending axones of the large and small pyramidal cells and of the polygonal cells of the deep layer. These axones become medullated and pass *(a)* to other convolutions of the same hemisphere—association fibres—these may be adjacent convolutions in the same lobe or distant convolutions in the same or other lobes; *(b)* through the corpus callosum to convolutions of the opposite hemisphere—these are also fibres of association, but are conveniently called commissural fibres; *(c)* to the internal capsule as fibres of the descending tracts (projection fibres).

(2) The ascending axones of cells situated in other parts of the nervous system, which are passing to their terminal arborizations among the cells of the cortex cerebri. These fibres are: *(a)* axones of cell bodies which are situated in other convolutions of the same hemisphere—*association fibres; (b)* axones of cell bodies which are situated in the convolutions of the opposite hemisphere—these pass through the corpus callosum—*commissural fibres; (c)* axones which have come through the internal capsule from cells situated in lower centres—*projection fibres;* these axones are passing to their terminal arborizations in the cortex.

Practical Study.

Vertical Section through the Human Cortex Cerebri—Motor Area. Stained by the method of Nissl. Mount in **balsam.**

(This section is for the study of cells alone, the fibres being unstained.)

Low Power.—Note the *layers of the cortex: (a)* the outer molecular layer, containing only a few nerve cells; *(b)* the broad layer of pyramidal cells, most of which are small or of medium size, some large pyramidal cells, however, being scattered among the small cells of the deeper part of the layer; *(c)* the layer of irregu-

lar or polymorphous cells situated next the white matter. Notice whether any of the very large pyramidal cells—cells of Betz—can be seen in the pyramidal layer.

Make a drawing of a thin vertical segment of the cortex showing the different layers and the number, size, shape, and arrangement of the cells.

High Power.—Make a drawing of several of the pyramidal cells of different sizes showing the nucleus, nucleolus, and the shape, size, and arrangement of the chromophilic bodies. Compare the arrangement of the chromatic substance in these cells with its arrangement in the anterior horn cells and in the cells of Purkinje. If you can find one of the cells of Betz, draw it and compare the arrangement of its chromophilic bodies with that of the chromophilic bodies in the cells of the anterior horn.

Transverse Section of One or Two Convolutions of the Human Cerebrum. Stained by Weigert's method. Mount in balsam.

Naked Eye and Dissecting Microscope.—Note that the *central core* or white matter of each convolution is stained dark blue or black, and that this merges into the more lightly stained or unstained *gray matter* which surrounds it.

(This section is for the study of fibres only, the cells being unstained.)

Low Power.—Note that the white matter is composed of medullated fibres which in the central portion of the convolution run parallel to one another, but which on reaching the edge of the gray matter radiate into the latter in a fan-like manner. These are projection, commissural, and association fibres intermingled, and which, being morphologically alike, are not distinguishable from one another. If the section contains two convolutions, some association fibres may be traced curving around the sulcus and connecting the two convolutions. Note in the molecular layer a few fine medullated fibres running parallel to the surface. These are the superficial tangential fibres. Deeper in the cortex are seen other fibres which pass in a direction at right angles to that of the main mass of projection fibres. These are known as deep tangential fibres. Note on the surface of the convolution the pia mater, if present.

Make a large outline drawing with the *naked eye or dissecting*

microscope, and with the *low power* fill in details showing the above arrangement of the fibres.

Transverse Section through the Cerebrum (and Basal Ganglia in some Sections) of a Young Mouse. Stained by *Golgi's silver method. The section has been mounted in balsam without a cover-glass, and care should be observed in the use of the high power.*

This section is for the purpose of giving a more complete picture of the neurones of the cortex than could be obtained from the previous sections, in one of which only the cells were stained, in the other only the medullated fibres. In this section the complete neurone, *i.e.*, the cell body and all its processes, is stained, thus giving a better conception of the relations between cells and fibres.

Low Power.—Observe the general structure of the section, the two hemispheres connected by a commissure, the *corpus callosum.* Note that the nerve cells and their processes are stained (impregnated) black. The number of neurones impregnated represents only a small proportion of the neurones in the specimen, the great majority of the neurones being unimpregnated and consequently invisible. Select a neurone in which the cell body and processes are completely stained and observe the following points: the shape of the cell body; its main or apical dendrite with its branches and their little knob-like projections or "gemmules"; other smaller dendrites coming off from the sides and base of the cell; the fine axone starting from the base of the cell and running a comparatively straight course. Note that the axone, unlike the dendrites, has few branches, and that these few come off approximately at right angles (collaterals). Observe the direction which the axone takes and follow it as far as possible in the section. It may be impossible to note all these points in any single neurone. If so, make use of several neurones in the study.

Make a drawing of *an entire neurone* showing the above details. Both high and low powers may be used in making this drawing.

Notice the fine fibres terminating freely in the cortex. These are mainly axones of cells situated in other parts of the gray matter of the nervous system, which have entered this part of the cortex to end in terminal arborizations among the cells.

Make a drawing of some of these terminations, and if possible show their relations to the bodies and dendrites of the cells.

Parts of urinary tubule
where found & functions.

Typical cell

Connective tissue classification

areolar variety

Stellate fixed.

Subcutaneous

Cartilage

Mucous Stomach 28 hrs

No difference between it &
intestine.

White blood cells.

Bronchi

Spleen.

Tongue { Filiform
Fungiform
Circumvallate }

Inf. Peduncle Cerebellum

Corpora restiform composed

of { Direct cerebellar tract
Fibres com from opposite side
Fibres from nucl. Goll &
Burdach of opposite side
Fibres from nucl. Goll
& Burdach of same side

7
13 days

$$7\,NaH_2(CO_2)_2 + NaH_2 =$$

$$CO_2 + NaH$$

13
1.003
9681

Sudermann
Affelgate 8
Burnham 10
Dreamer 9
Offenheimer 8
Wright 9½
Friedman 8
Rettenberg 9
Voltaire 6
Smith 10
Sloan 9
Truesdell 9
Pichard 10
Vail
Peace
Corcoran
Spiegelberg
Handy
Connelly
Phelps
Dubois
$2,500
Dreyer 9
Leib 9
Harrington
Tyrone
Kingsley
Welling
Sommer

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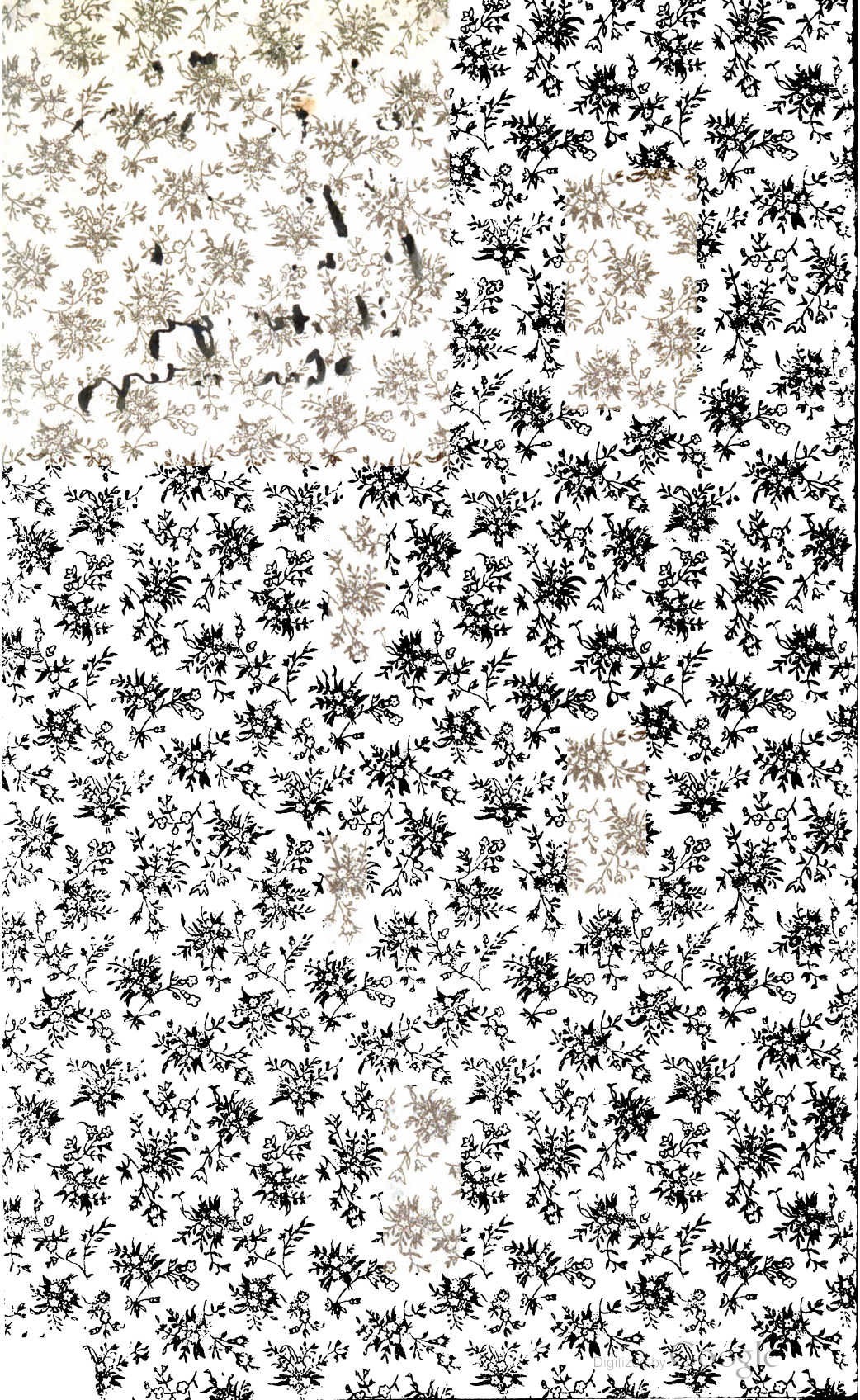

Phy. Chem.
Bact.
Physiol.
Path. Anat.
Histology
Pharm.